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Cover Photograph: Indian Pink (*Spigelia marilandica*). The photo was taken on Choccolocco Mountain in Mountain Longleaf National Wildlife Refuge. The plant is common in forests across much of Alabama.

Photo is courtesy of: Bill Garland, Biologist.

Editorial Comment:

On behalf of the Alabama Academy of Science, I would like to express my gratitude and appreciation to the following for their valuable contributions in reviewing the manuscripts of this issue:

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Safaa Al-Hamdani
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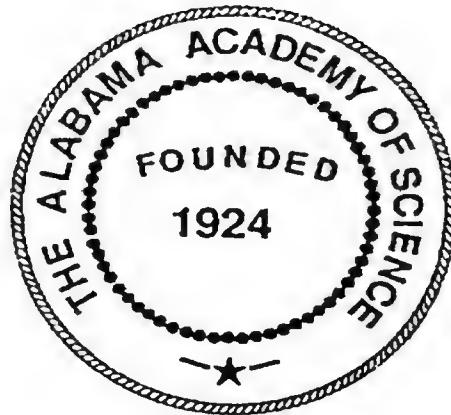
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CONTENTS

ARTICLE:

Bryan S. Arwood, Robert W. Peters, and Robert A.....	1
William E. Kelly.....	14
Nicole Martin, and Mark M. Lanier.....	26
David M. Ponder and Safaa H. Al-Hamdani.....	44

MEMBERSHIP LIST.....	54
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THE EFFECTIVENESS OF ADVANCED OXIDATION TECHNIQUES IN DEGRADING ETHYNYLESTRAZOL IN WATER

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ABSTRACT

Estrogens, both natural and synthetic, are excreted by females. Wastewater treatment plants (WWTPs) frequently fail to remove steroid compounds completely before discharge into receiving waterways. Studies frequently detect estrogenic endocrine-disrupting chemicals (EDCs) in receiving water in concentrations sufficient to affect aquatic organisms living in those waters. Thus, there is a need to improve the effectiveness of wastewater treatment methods in removing EDCs. A treatment method currently being proposed uses various methods of oxidation, known as advanced oxidation techniques (AOTs), to more effectively break down environmental estrogens in wastewater. This study investigated the effectiveness of three AOT methods: sonication, UV photolysis, and ozonolysis. Deionized water was spiked with the synthetic estrogen, 17 α -ethynylestradiol (EE2), a synthetic estrogen found in birth control pills, and then was treated for various lengths of time. The treated samples were analyzed using reverse phase high pressure liquid chromatography to compare the amount of remaining EE2 with that in the untreated spiked water sample and were tested for residual estrogenic activity using a yeast estrogen screening assay.

INTRODUCTION

In the past, concerns about chemicals in the environment tended to focus on toxic and carcinogenic effects. Recently, however, the USEPA and others have expressed concern about environmental chemicals with the potential to interfere with endocrine functions of humans and wildlife. An endocrine disrupting compound (EDC) can be defined as “an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior” (Kavlock et al., 1996). Furthermore, the European Union (EU) has suggested that EDCs can be placed into three broad classifications: 1) synthetic hormones or chemicals designed to deliberately alter the endocrine system, 2) natural compounds and their metabolites, and 3) chemicals which, by chance, disrupt the endocrine system (Shi and Kujawa-Roeleveld, 2007). The most potentially disruptive of these are the synthetic hormones, which are intentionally designed to affect the endocrine system at low concentrations, typically for therapeutic purposes.

Since the early 1920’s, hormone therapy has been used to treat hormone imbalances in patients; however, a more common current use of hormones is in oral contraceptives. In 2006,

Degrading Estrogen with Advanced Oxidation Techniques

17 α -ethynodiol (EE2), a synthetic estrogen and active ingredient in birth control pills, was the most prescribed drug in the world (Shi and Kujawa-Roeleveld, 2007; Gunnarsson et al., 2009). In the Netherlands, 43% of females of reproductive age regularly take pills containing EE2 for contraceptive use (Shi and Kujawa-Roeleveld, 2007). A single birth control pill contains between 25-50 μ g of EE2, and the average daily dosage is 35 μ g (de Rudder et al., 2004; Shi and Kujawa-Roeleveld, 2007; Saaristo et al., 2009; Hashimoto and Murakami, 2009). Thus, it is not surprising that EE2 is ubiquitous in the environment. It has been detected in wastewater, surface waters, sediments, groundwater, and potable water sources worldwide, potentially causing detrimental effects on the health of humans and wildlife (Snyder et al., 2008; Liu et al., 2009).

EE2 is able to bind to the estrogen receptors α and β (ER α , ER β) and initiate a genetic response leading to the transcription of specific proteins (Weyer and Riley, 2001; McLachlan et al., 2006). This has been demonstrated by studies, for example, with the western mosquitofish (*Gambusia affinis*) and the sand goby (*Pomatoschistus minutus*), where it has been shown that as little as 0.1 ng L^{-1} can induce expression of the estrogen-dependent gene for vitellogenin (Vtg) (Angus et al., 2005; Saaristo et al., 2009). Vitellogenin is an egg yolk precursor protein synthesized in the liver and excreted into the blood and specifically taken up by developing eggs. The presence of Vtg in the blood of males or sexually immature individuals is a useful biomarker of exposure to estrogenic chemicals. Both ER- α and ER- β are highly conserved throughout the vertebrates. Thus, although EE2 was designed to act as an estrogen in humans, it acts as an estrogen, and causes endocrine disruption, in a wide range of vertebrates. For example, ovotestes (a biomarker of feminization) and reduced male copulatory behavior was seen in male Japanese quail exposed to EE2 (Elbrecht and Smith, 1992; Berg et al., 1998). Similar results have been documented in reptiles, amphibians, fish and gastropods in nature (CSTEE, 1999). It appears that EE2 may potentially act as an endocrine disruptor in invertebrates as well. ER orthologs have recently been detected in some invertebrates such as the mollusk, *Aplysia californica* (Carroll et al., 2008).

Endocrine disruptors do not just affect the reproductive organs. Another mounting concern is the effects of EE2 on sexual behavior and courtship activity. It has been shown with the roach fish (*Rutilus rutilus*) that exposure to EE2 can lead to increased recruitment of ERs or even total feminization of a male if sexual differentiation has not yet occurred (Lange et al., 2009). Early receptor recruitment eventually leads to increased estrogen sensitivity at a later life stage (Lange et al., 2009). Disruption of male sexual behavior due to EE2 exposure has also been documented in the sand goby (Saaristo et al., 2009). Exposure to EE2 via the water led to a decline in sand goby courtship behavior by males and the eventual breakdown of the sexual selection processes in the population (Saaristo et al., 2009). Behavior disruption has also been documented in the zebra finch (*Taeniopygia guttata*), where birds fed estrogen-spiked food pellets showed abnormal nesting behavior. Because nest building behavior in *T. guttata* is sexually dimorphic, researchers were able to observe the degradation in nest integrity when estrogen was present in the male diet (Rochester et al., 2008). However, the long term impacts in males of exposure to environmental EE2 are still unknown (Liu et al., 2009; Saaristo et al., 2009). It is because of both the known and unknown effects that many researchers feel that the best solution to mitigating the effects of EDCs lies in removing them from, or greatly reducing their concentration in, the environment. For most EDCs, the most effective means of reducing their occurrence in the environment is through better wastewater treatment methods (de Rudder et al., 2004; Snyder et al., 2008; Liu et al., 2009).

Upon excretion from the body, EE2 can persist in waterways for 20-40 days, depending on environmental conditions (Panter et al., 1999). Moreover, the excretion of EE2 occurs every 21 out of 28 days in females following the typical oral contraception regime (Shi and Kujawa-Roeleveld, 2007). Of the EE2 ingested, up to 50% is excreted in urine while 30% is excreted through feces (Shi and Kujawa-Roeleveld, 2007). The remaining 20% undergoes de-ethynylation and is converted into 17β -estradiol (E2) and estrone (E1) (Shi and Kujawa-Roeleveld, 2007). Although approximately 64% of the EE2 is excreted in a biologically inactive conjugated form (with a glucuronide or sulfate), it may become reactivated if the conjugate is removed through microbial activity (Shi and Kujawa-Roeleveld, 2007; De Gusseme et al., 2009; Panter et al., 1999). Its environmental persistence and high estrogenic potency make EE2 one of the most important contributors to the overall estrogenic activity in effluents released from wastewater treatment plants (WWTPs) (Hemming et al., 2004; Gunnarsson et al., 2009). For these reasons, many researchers have concluded that it is the EDC of greatest environmental concern (de Rudder et al., 2004; Hemming et al., 2004).

While current WWTPs are equipped to efficiently remove various water soluble organic substances, the removal of EE2 and other pharmaceuticals with low solubility in water is often less complete (de Rudder et al., 2004). EE2 is extremely hydrophobic, with a solubility of only 4.8 mg L^{-1} at 20°C . In contrast, 17β -estradiol has a solubility of 13 mg L^{-1} . As a result of their inability to completely remove estrogens from wastewater, WWTPs have been identified as significant sources of environmental EE2 (Jürgens et al., 2002; de Rudder et al., 2004; Hashimoto and Murakami, 2009). Concentrations of EE2 as high as 28 ng L^{-1} have been detected in domestic wastewaters, and as high as 106 ng L^{-1} have been detected in samples from WWTP effluents where contemporary water treatment methods were being implemented (Clara et al., 2005).

Three methods are typically employed in removing organics from WWTP effluent: physical removal, biodegradation, and sedimentation (Liu et al., 2009). These treatment regimes differ in their efficiency of EE2 removal and required processing time. It has been noted that, under these traditional treatments, EE2 is not removed as effectively as other environmental estrogens, in particular natural hormones (Hashimoto and Murakami, 2009). For example, in a recent study, EE2 was the most common estrogen found in the effluents being discharged from 13 WWTPs sampled throughout Europe (Janex-Habibi et al., 2009). Moreover, it appears that when traditional biodegradation techniques are supplemented with additional effluent polishing procedures, EE2 can still take an average of 8.92 days to degrade (de Rudder et al., 2004; De Gusseme et al., 2009).

Because of the rather extensive treatment time required to effectively remove EE2 from wastewater, there exist questions concerning the capability of traditional wastewater management strategies to deal with the problem of estrogens and pharmaceuticals (De Gusseme et al., 2009; Gunnarsson et al., 2009; Hashimoto and Murakami, 2009). With respect to physical methods of EE2 elimination, such as using activated carbon (AC) and membranes, studies have shown that adsorption and filtration capacity decreases over time (Iwasaki et al., 2002; Liu et al., 2009). Alternatively, advanced oxidation treatment (AOT) methods may be preferable because of their effectiveness in degrading hormones and pharmaceuticals in wastewater and the low environmental risk associated with their operation (Liu et al., 2009).

AOTs act by mineralizing target compounds into carbon dioxide (CO_2), or a breakdown product via an oxidation reduction (redox) reaction (Fontanier et al., 2006; Rosal et al., 2008; Liu

Degrading Estrogen with Advanced Oxidation Techniques

et al., 2009). Current AOT procedures being tested include ultraviolet (UV) light, hydrogen peroxide (H_2O_2), and ozone (O_3), usually in a specific combination in order to treat wastewater of a known character. Removal efficiency rates as high as 90% have been observed when using UV photolysis with H_2O_2 to remove various EDCs in scaled plant studies (Liu et al., 2009). With respect to EE2, this redox reaction creates a hydroxyl radical which behaves as an electron acceptor weakening or degrading the structure of EE2 and possibly allowing for further bacterial degradation. Because bacterial degradation is currently employed during various stages in wastewater treatment, AOT methods can be integrated into existing WWTP operations.

The objective of the present study was to investigate the effectiveness of two proven AOT techniques in combination (UV and ozone), along with sonication, in degrading EE2 in water. We hypothesized that the use of full spectrum UV photolysis and ozonolysis together would result in a synergistic effect. UV photolysis alone has been shown to degrade EE2 by up to 70%, while ozone has exhibited a maximum degradation of 53.9% (Liu et al., 2009). We also hypothesized that, by supplementing these AOT methods with sonication, additional degradation would occur through physical means (Hickenboth et al., 2007). Sonication produces fine microbubbles. The microbubbles grow to a critical size and then implode, releasing a considerable amount of energy, with pressures in the range of 500-1000 atm and temperatures of approximately 5000°K around the collapsing interface. As EE2 is subjected to sonication, the alternating compression cycles created by the condensed sound waves repeatedly stress the molecular bonds, leading to the breakdown of the molecular structure and a destruction of estrogenic activity.

MATERIALS AND METHODS

17 α -ethynylestradiol Solution

A 1 mM stock solution of EE2 [1, 3, 5(10)-estratrien-17 α -ethynyl-3,17 β -diol, Steraloids, Inc., Newport, RI, USA] was prepared by dissolving 0.0030 g of EE2 in 10 ml of absolute ethanol. A fresh stock solution was prepared daily. Samples for treatment (1 μ M) were prepared by bringing 1 mL of the stock EE2 solution up to 1000 mL with deionized water.

Advanced Oxidation Techniques and Exposure

The treatments used in this study are shown in Table 1. Treatment times were 10, 20, 30, 40, 50, and 60 minutes. Initial treatments using single advanced oxidants were done on one or two samples each. After it became apparent that treatments using single oxidants were not effectively reducing estrogenicity, it was decided to focus on a combination of all treatments. For each combined oxidative treatment time, five samples were exposed to the treatment, and three samples (controls) were unexposed. All treatments were performed in 1 L acid-washed KIMAX heavy duty beakers. A beaker containing the EE2 solution was placed into the UV photo reactor along with the sonication applicator. A silicon tube was placed into the beaker to act as a conduit for ozone. For the single AOT exposure experiments, the protocol was the same except that each AOT was administered individually.

Solid-phase Extraction

The treated EE2 solutions were passed through solid-phase extraction cartridges (SPEC) (10 mL, C-18; Varian, Walnut Creek, CA, USA) which had been primed by passing 1 mL methanol and 1 mL deionized (DI) water through them. The columns were then stored at -13°C until elution. Organics were eluted from each SPEC column with 6 mL of HPLC grade methanol

(99.9% pure) and collected in an acid-washed glass vial. Extraction efficiency was determined to be 88.9%. Each sample was then dried under nitrogen and stored at 4°C if HPLC could not be performed immediately.

Table 1. Advanced oxidation treatments used in this study.

Treatment	Amount
Ozone	100 ppm (2.11×10^{-9} mg m ⁻³)
UV light	16 50 W bulbs, 80 W total
Sonication	20 KHz
Ozone + UV +	as above
Sonication	

High Performance Liquid Chromatography and Steroid Identification

Reverse phase HPLC was performed on a Perkin-Elmer LC-95 system (PerkinElmer, Waltham, MA, USA) with a 100 × 4.6 mm, C-18 Varian Chromosep column. Compounds eluting from the column were detected by a Perkin Elmer UV detector at 235 nm. The solvent gradient was modified from that used by Jenkins et al. (2000). It started with a 1:1 water: acetonitrile mixture and progressed linearly over a period of 10 minutes to 100% acetonitrile, where it was held for a further 10 minutes. Peak areas were determined using TC NAV HPLC analysis software (Perkin-Elmer). EE2 from the untreated standard solution had an average retention time of 5.85 minutes and an average peak area of 6.44×10^6 μ V min. The area under the EE2 peak of a treated sample was compared to the average area under the peak of the control samples run on the same day to establish a percent of the original area under the peak still remaining. Each sample was analyzed by HPLC three times. Analytes were recovered, inventoried, and disposed of in accordance with university Occupational Health and Safety standards.

Yeast Estrogen Screen

Yeast estrogen screen (YES) assays were performed in order to quantify the amount of estrogenic activity remaining after EE2 had been treated with the combined AOT methods. The protocol was modified from Fox et al. (2008). The YES assay used a strain of genetically engineered yeast cells, generously provided by Charles A. Miller III, with a plasmid containing the gene for the human estrogen receptor β , an estrogen response element and the bacterial enzyme β galactosidase gene, which served as a reporter.

The cells used in the YES assay lack the ability to synthesize the amino acid tryptophan (TRP). The cells were grown in a yeast nitrogen base (YNB) medium containing all essential free amino acids (AA) except TRP. Because the plasmid contains a functional *trp1* gene, the tryptophan-lacking medium contraselects any yeast cells that have lost the plasmid. At the beginning of an assay, a single colony of yeast cells was relocated from solid media to 3 mL of liquid media consisting of 100x AA TRP-glucose YNB and incubated overnight. The overnight culture was allowed to grow to saturation and subsequently diluted to 2×10^6 cells/mL in the same liquid media. Cell densities were established by the absorbance at 600 nm using a Bio Rad Model 550 microplate reader. The culture was then grown for 4-5 hours at 30°C, allowing the

Degrading Estrogen with Advanced Oxidation Techniques

cell densities to reach 1×10^7 cells/mL. Then a final dilution was done using 100x AA TRP-Galactose YNB liquid media in order to obtain a working cell density of 4×10^5 cells/ mL.

Yeast cells were exposed to a series of known concentrations of E2 serving as controls and to AOT-exposed samples (at one half their original concentration) in sterile 96 well u-bottom culture plates (Falcon, Franklin Lakes, NJ USA). There were four replicates for each sample. In each well, 200 μ L of cell culture and 2 μ L of control or AOT-treated EE2 sample were added. During exposure, the yeast cells were incubated for 18 hours at 30°C.

After incubation, cell density was measured using the microplate reader at 600 nm. Next, cells were lysed by adding a lac z buffer containing the chromogenic β galactosidase substrate ortho-nitrophenyl- β -galactoside (ONPG). The lysed cells were incubated with the substrate for 15-35 minutes at 37°C. During this time, the β -galactosidase released from the lysed cells cleaved the ONPG to produce ortho-nitrophenol, which is yellow. The ONPG incubation was visually monitored and, when a yellow hue was observed in the positive control wells, the reaction was stopped with sodium carbonate (1M). The intensity of the yellow color was measured with the microplate reader as optical density at 405 nm.

The amount of chromogenic product in each plate well was determined by calculating lac z units, which are related to the intensity of the yellow color, using the following formula:

$$\text{lac z units} = \frac{1000 \times (\text{ABS}_{450 \text{ sample}} - \text{ABS}_{405 \text{ control}})}{\text{min} \times \text{mL} \times \text{ABS}_{600 \text{ sample}}}$$

where ABS₄₀₅ and ABS₆₀₀ are the absorbances of the wells at 405 and 600 nm, respectively, min is the development time in minutes, and mL is the volume of the exposed cells and medium that was lysed with lac z buffer (Fox et al., 2008).

A plot of lac z units vs. log (E2 concentration) was used as a standard curve to quantify the estrogenic activity of the treated samples, expressed as E2 equivalents (EEQs).

Statistical Methods

For analysis of HPLC data, all EE2 peak areas of treated samples were converted to a percentage of the area of the untreated control samples for the same date. All summary statistics are expressed as mean \pm standard error. Treatments using a single AOT were not compared statistically since only one or two samples were treated at any time period. For the samples treated with multiple AOTs, at each time period, the difference in percent EE2 degradation between the treated samples ($n = 5$) and the untreated controls ($n = 3$) run on the same day was tested using a one-tailed non-parametric Mann-Whitney U test. For all statistical tests, the cutoff for significance was $p \leq 0.05$.

For samples treated with combined AOTs, the decrease in estrogenic activity as measured as EEQs in the YES assay (y) over treatment time (x) was modeled using a first-order exponential decay formula $y = ae^{-bx}$ where a = the initial EEQ value and b is the reciprocal of the decay constant, λ .

RESULTS

HPLC Analyses

The experiments where aqueous EE2 samples were treated by AOTs (ozone and UV) one at a time ($n = 1$ or 2 per treatment) showed high variability, but no discernable reduction in peak area even after 60 minutes of treatment (Figs. 1 and 2). When the combined AOT techniques were applied ($n = 5$ per treatment), significant decreases in mean peak area over time were seen (Fig. 3). The mean peak areas of all the treatments > 20 min were significantly less than their untreated controls ($n = 3$ controls per treatment).

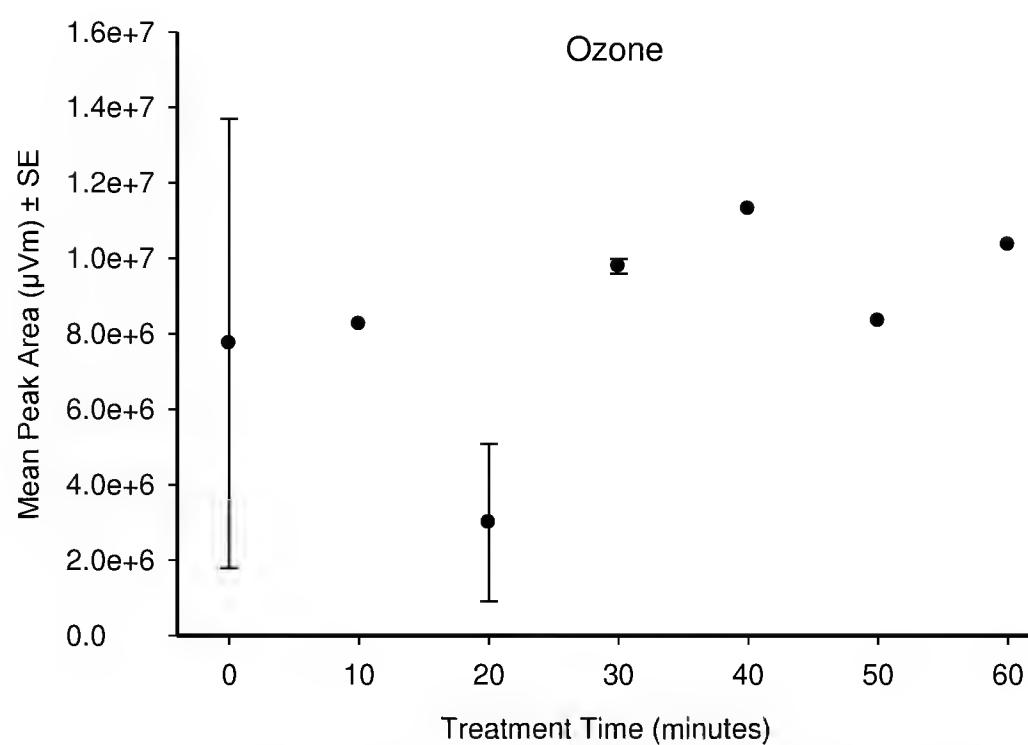


Figure 1. Ozonolysis of EE2. Remaining peak area after EE2 was exposed to 100 ppm ozone for up to 60 minutes. Points without error bars were from a single replicate. At 0 min, $n = 2$; at 20 min $n = 5$, and at 30 min $n = 2$.

Inspection of the chromatograms for the treated samples (Fig. 4) showed an increase in the number of peaks up field of the parent EE2 peak, compared to untreated controls. These represent more polar compounds, presumably produced by the breakdown of EE2 during treatment.

Degrading Estrogen with Advanced Oxidation Techniques

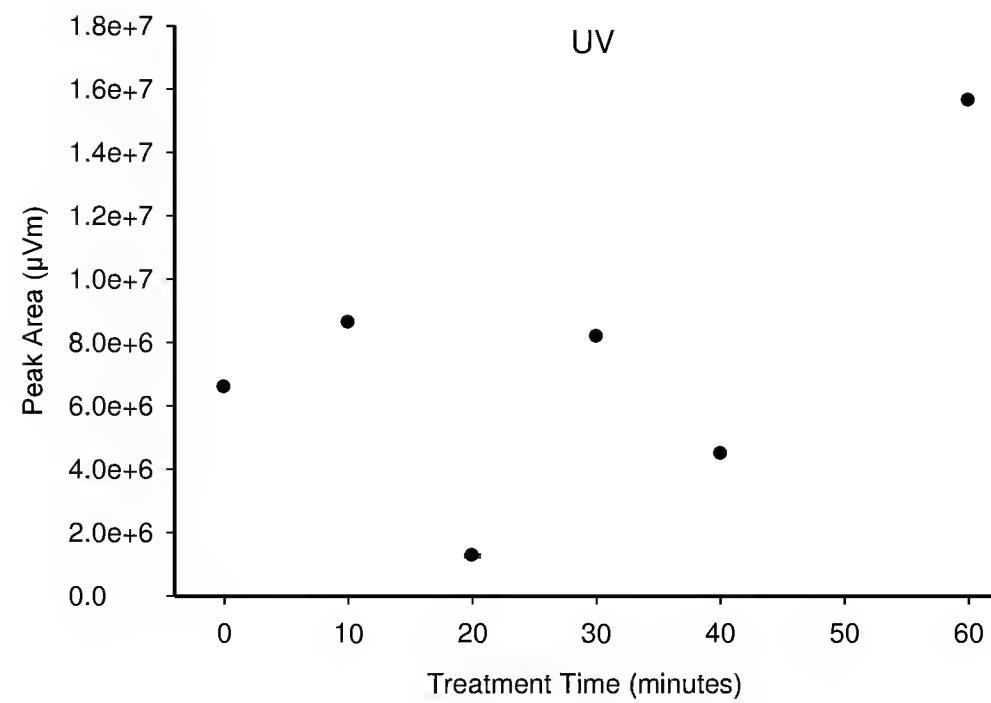


Figure 2. UV photolysis of EE2. Remaining peak area after EE2 was exposed to UV light for up to 60 minutes. Each point represents the results of a single treatment except 20 min where $n = 4$. The SE of the 20-min mean is covered by the plotting symbol.

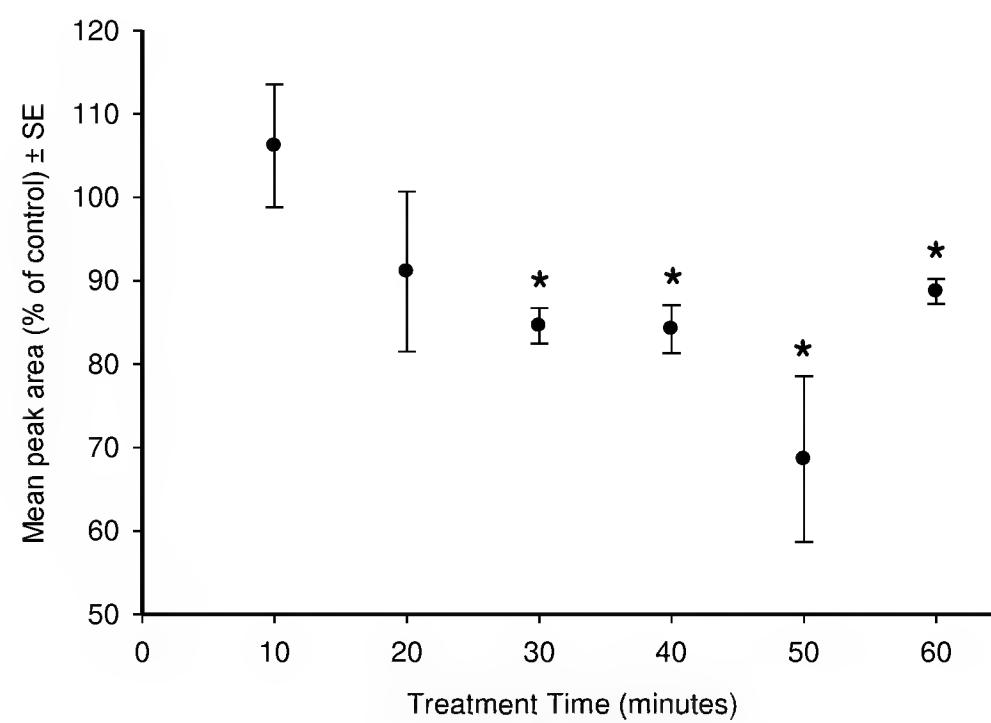


Figure 3. Percentage of Peak Area Remaining after Combined Treatment with Sonication, Ozone and UV. The mean peak area of the treated sample at each time point is shown as a percentage of the mean peak area of the untreated control analyzed on the same day. Each mean is based on a sample of $n = 5$. Means shown with asterisks are significantly less than their respective controls ($p_{1\text{-tailed}} < 0.05$, Mann-Whitney U-tests).

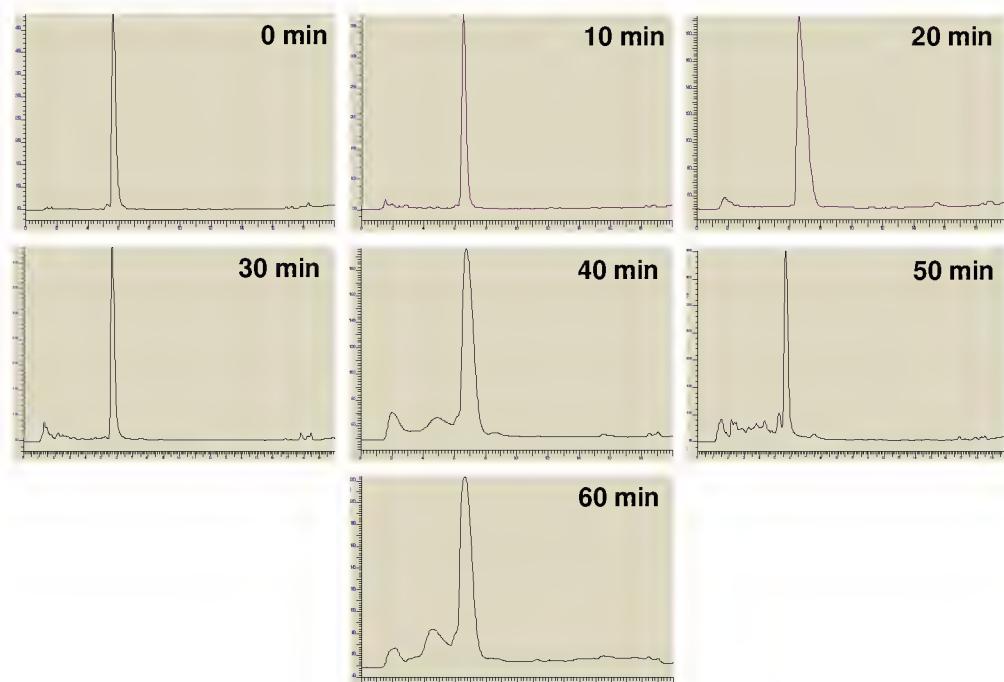


Figure 4. An increase in up field peaks over treatment length. As treatment time progressed, an increased number of chromatographic peaks can be observed to the left of the parent peak.

YES Analysis of Remaining Estrogenic Activity

The amount of estrogenic activity remaining in the samples treated with combined sonication, ozone and UV declined steadily with time (Fig. 5). The rate of degradation in estrogenic activity, as measured by the YES assay, was fit to a first order exponential decay model (see Statistical Methods, above, and also Fig. 5). The estimated parameter values were $a = 2.00 \pm 0.73$, and $b = 0.06 \pm 0.02$. Due to scatter in the data, the fit was not very tight; the adjusted r^2 was 0.37.

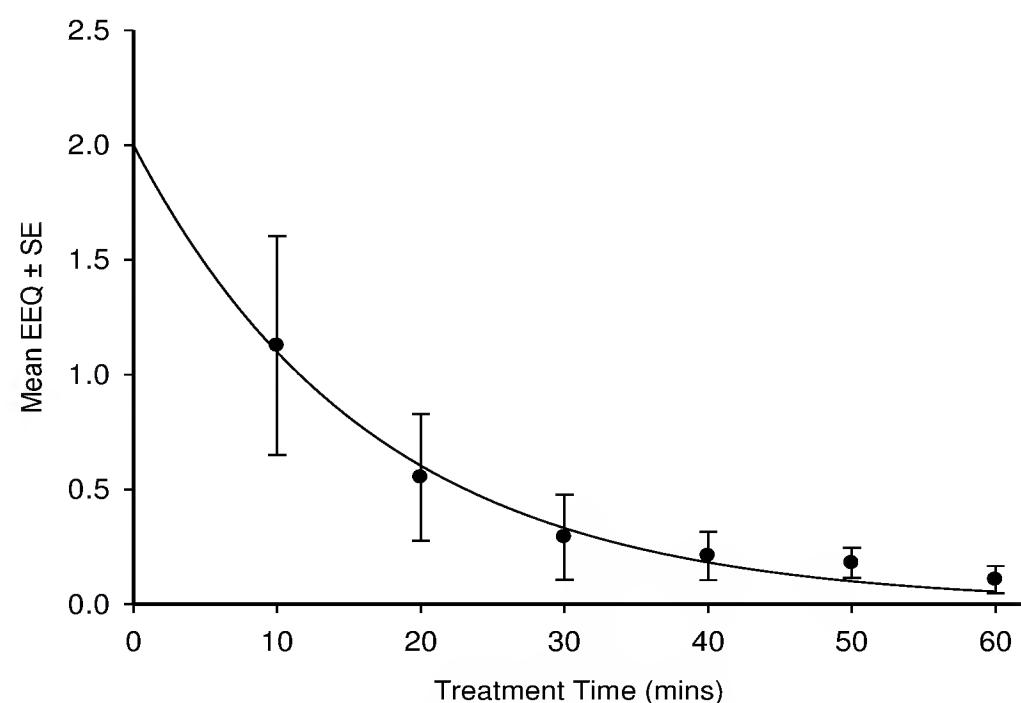


Figure 5. Mean residual estrogenic activity (in EEQs, equivalent to nM of 17β estradiol) of EE2-spiked water samples treated with combined sonication, ozone and UV. The line is the best fit to the two-parameter exponential decay model, $y=ae^{-bx}$.

DISCUSSION

The results of the current study support our hypothesis that combined treatment with AOTs and sonication can effectively reduce estrogenic activity in aqueous solutions. This may be used as a possible pre-treatment in WWTPs prior to microbial treatment. In addition to immediately

Degrading Estrogen with Advanced Oxidation Techniques

reducing estrogenic activity, we suspect that the AOTs and sonication will cause sufficient damage to the estrogen molecules that they will be more susceptible to bacterial degradation during the secondary treatment process.

Typically, AOT methods have consisted of UV-photolysis, ozonolysis, and H_2O_2 exposure (Lin and Reinhard, 2004; Liu et al., 2008). Of these, UV-photolysis and ozonolysis appear to be the most feasible financially because they can be generated economically onsite. Additionally, when employing H_2O_2 in a wastewater treatment regime, the pH must be continuously monitored in order to achieve efficient removal of contaminants (Esplugas et al., 2007; Rosal et al., 2008). Given that UV disinfection is already being used in many WWTPs to disinfect the effluent, it may be possible to incorporate it into a dual role (through wastewater recycling) and to combine it with ozone and sonication. When proposing additional unit processes to a WWTP, one must consider the effectiveness of the treatment method(s) and the amount of processing time required to reach the accepted concentrations in the effluent (Iwasaki et al., 2001; Liu et al., 2008). One review comparing the removal of endocrine disruptors from water effluents in 44 studies indicated that, in order to eliminate EE2 from wastewater, a UV photoreactor would have to employ a UV lamp between 152-250 W (Esplugas et al., 2007). In a recent study (Coleman et al., 2005), the effects of UV photolysis on steroid estrogens were examined. It was noted that UV photolysis (using a 152 W UV lamp) effectively reduced EE2's estrogenicity by up to 100% at treatment times exceeding one hour. Our preliminary results suggested that UV exposure at 80 W (the maximum that we had available) had no significant effect on EE2 concentrations or activity even after an hour of treatment (Fig. 2). In the current study, however, we were able to achieve significant reduction in estrogenicity using an 80 W UV lamp at 50 minutes of exposure in combination with O_3 and sonication (Fig. 5).

Ozone can be used to degrade contaminants in wastewater directly or with the aid of a catalyst (Huber et al., 2004; Fontanier et al., 2006). However, pH must be carefully maintained to achieve optimal removal of organic constituents when certain catalysts (e.g. TOCCATA[®]) are used. Additionally, shifts in pH can upset the microorganisms used in wastewater treatment. Chemical catalysts were not tested in the current study.

One study (Huber et al., 2004) has shown that O_3 by itself (at 100 ppm) can effectively reduce the estrogenic activity of EE2 after 20 minutes of exposure without the aid of a catalyst. However, results of the current study, also using 100 ppm O_3 , are not consistent with those of Huber et al. (2004). There was no detectable decrease in EE2 peak areas of O_3 -treated water samples when treated for up to 60 minutes (Fig. 1). Possible explanations for our different results include the absence of a catalyst during ozonolysis and/or not maintaining a constant pH during ozone exposure. However, results of the current study do suggest a synergistic effect between the three treatments methods, without the addition of any catalytic additives, compared to the same treatments used in unison (Figs. 1-3, 5).

When determining the effectiveness of AOT methods at breaking down EE2 in aqueous solution, it is useful to analyze the extent to which degradation has occurred using HPLC. However, the method does have limitations. The AOT methods need not destroy the EE2 molecules to be effective. All that is necessary is that sufficient molecular alterations be produced so that estrogenic activity is reduced or eliminated. By altering the molecular structure of EE2 even slightly, it is possible to decrease its affinity to the ER (Heldring et al., 2007). If the AOT treatment caused some breakdown of the EE2, the products would consist of smaller, more polar fragments. This was supported by the results of our HPLC analyses. As the concentration

of acetonitrile increased over time, the more polar (EE2) breakdown products eluted faster, and thus had shorter retention times relative to the nonpolar parent EE2 molecule. This can be seen in Fig. 4, where the number of visible chromatographic peaks, mostly between 2 – 4 min, increased as a product of longer treatment exposures.

Because reduction of estrogenicity was the biological end point of interest in the current study, we employed a YES assay to measure residual estrogenic activity subsequent to the treatments. Whereas reverse phase HPLC indicated no apparent trend of decrease in the concentration of remaining EE2 after 20 minutes of AOT exposure (Fig. 3), the YES assay showed a significant downward trend in estrogenicity with time of treatment (Fig. 5). These results support our hypothesis that the AOT methods altered the configuration of EE2 enough to impede its interaction with human ER β .

The current study evaluates the effectiveness of AOT methods in breaking down a steroid in a very simple system, i.e. a single steroid in deionized water. Real wastewater is a highly complex mixture of constituents that may affect the effectiveness of AOT treatment. The current study chose to evaluate EE2 since it is a common estrogenic constituent in domestic wastewater, is environmentally persistent and is biologically very active. However, wastewater will contain a variety of natural steroids and synthetic chemicals with estrogenic activity. A logical follow up to the current study would be to evaluate the effectiveness of AOT treatments in reducing the estrogenic activity of various mixtures of steroids and other estrogenic chemicals. Since the composition of wastewater should be somewhat more uniform post treatment, it would be reasonable to consider adding AOT to the process after most of the organic constituents have been removed or reduced by conventional treatment.

In conclusion, the present study indicates that treatment using the combined AOT methods (sonication, O₃ and UV light) significantly reduces both the concentration and estrogenic activity of EE2 in aqueous solution. This is potentially useful as EE2 is one of the most potent environmental estrogens present in the effluent of WWTPs. Subsequently, it would be logical to devise a practical add-on system that could be installed in existing WWTPs in order to improve the efficiency of removal of estrogenic compounds from wastewater.

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JUSTICE REVISITED: THE CATALYST OF THE VOTING RIGHTS ACT OF 1965

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ABSTRACT

The Voting Rights Act of 1965 was an important result of activity in Alabama that received a great deal of publicity in this country and abroad. It resulted in part from the famous Selma to Montgomery March in 1965, and the horrific events on one day commonly known as “Bloody Sunday”. Yet, why did the march take place? What caused so many people to stand up for a right and be subjected to horrendous beatings by state troopers? This author contends that one major reason was the shooting of a civil rights activist by a white Alabama state trooper during that turbulent year. It may not be the only reason, but it did seem to be a major reason. Perhaps the shooting represents an example of “path dependence” in the sense that where we are today is a result of an event that occurred in the past. The shooting may also be an example of the effect that a martyr has in bringing change to a society. In fact, Jimmie Lee Jackson is viewed as a martyr near his home town. The shooting occurred more than forty years ago, and for a number of years the state of Alabama had been seeking to prosecute the trooper for this act. This article examines the events of the shooting and identifies a number of factors that were important in the prosecution. Information about the event was obtained from various newspapers, but the author was fortunate to have obtained a declassified Federal Bureau of Investigation report of the shooting. The file is more than 150 pages long. It contains copies of actual reports of numerous interviews in Alabama conducted by agents of the Bureau regarding the shooting. It was quite helpful in clarifying some newspaper articles about the shooting.

INTRODUCTION

The famous “Selma to Montgomery March” in 1965 is one of the major events in the civil rights movement during the latter part of the 20th century. Not only did it galvanize the nation into recognizing racial inequality in the United States, but it also resulted in the passage of the 1965 Voting Rights Act, which opened the door of political participation for thousands of minorities and propelled many of them into political office. Today, its political effects are apparent in many southern states as government officials are often minority citizens, especially on the local and county levels. This impact is particularly evident in areas of a state where African-Americans represent a high percentage of residents.

The Selma to Montgomery march itself was motivated by a number of reasons. Certainly one of the most obvious reasons is the shooting of Jimmie Lee Jackson by then Alabama State

Voting Rights Act of 1965

Trooper James Bonard Fowler. Mr. Fowler was indicted regarding the shooting a number of years ago by a state grand jury and could possibly stand trial for the shooting. The indictment in 2007 of the former Alabama trooper received considerable state and national attention most likely for its connection to the famous Selma to Montgomery march in 1965. In addition, the length of time from indictment to the conclusion of the case in late 2010 was long.

A “Cold Case” Reopened

The death of Jimmie Lee Jackson is one of a number of cold cases involving violence against civil rights workers that are being reopened. These attempts may be due to a number of factors such as “the growing clout of black elected officials in the South, the appearance of a new generation of Southern prosecutors eager to see that racially explosive era behind them, and the need of aging witnesses and perpetrators to clear their consciences before they die, according to civil rights groups and police” (Kemper, 2006).

Granted, many of these crimes occurred decades ago, but today those with information about these crimes may be more willing to come forward because they are less fearful of negative consequences for doing so. Thus, they may be more willing to provide information to authorities that could help in the prosecution of civil rights violators. In addition, some negative forces such as the Ku Klux Klan have declined, the social climate has changed, and the federal government is taking more action to help in such cases. Mark Vukelich, head of the FBI’s civil rights division, believes that the passing of time could be an asset to investigators concerned with cold case civil rights cases: “The thing that works to our advantage is the fact that attitudes change ... “I think it’s a bigger ally than the forensic aspect” (Kemper, 2006).

However, it is also possible that older individuals who experienced the negative effects of the civil rights protests and related activities of the 1960s have forgotten many incidents, remember them with less clarity and certainty, or do not want these matters brought up today. Nevertheless, there are still many individuals who do remember the events and would like to see some legal action taken against those who were responsible for serious crimes perpetrated against civil rights activists of the period.

Since 1994, there has been a series of trials involving individuals accused of crimes relating to civil rights activities. Some of these prosecutions have been referred to as “a lot of little Nurembergs” by writer David Halberstam (Kemper, 2006). Examples of such cases would involve the bombing of the Sixteenth Street Baptist Church in Birmingham in 1963, the assassination of Medgar Evers in 1963, and the murder of young civil rights workers in Mississippi in 1964 (Kemper, 2006). The case involving the death of Jimmie Lee Jackson is a fine example of a cold case being revived in this country and it is an important case regarding the civil rights movement.

How the Case Began

The shooting of civil rights worker Jimmie Lee Jackson occurred in a Marion, Alabama, a small town in an area commonly referred to as the Black Belt of Alabama. The year was 1965—a year characterized by much social strife in the state. James Bonard Fowler was one of a number of Alabama troopers sent to Marion by Alabama authorities in response to civil rights activities. One such activity included a nighttime march by hundreds of demonstrators from the Zion United Methodist Church to the local jail, where they had planned to sing freedom songs. The march was motivated by the fact that a civil rights leader was being held in the local jail.

However, between the demonstrators and the jail stood a contingent of law enforcement officers including Alabama state troopers (Fleming, March 2005). According to troopers present the night of the shooting, the marchers disobeyed orders to disperse. Sometime during the confrontation between law enforcement officials and the marchers the street lights went out. It has been alleged that after the lights went out, some troopers were struck by bricks and bottles. (Associated Content—May 2007). Law enforcement personnel reportedly used clubs to force the marchers back to the Zion Methodist Church. However, some of the marchers including Jackson ran from law enforcement officials into Mack's café, a restaurant for the local black community. A group of state troopers, including James Bonard Fowler, followed them into the restaurant.

The Shooting of Jimmie Lee Jackson

Accounts differ about the shooting of Jimmie Lee Jackson in Mack's café. One account paints a negative view of the troopers in the café and the shooting of Jackson by Fowler, noting that as the troopers entered the café, they started overturning tables and hitting people. Among those injured were Cager Lee, the grandfather of Jimmie Lee Jackson and Jackson's mother Viola Jackson. It is alleged that when Jackson attempted to help his mother, he was shot by an Alabama trooper (Fleming, March 6, 2005). This view is supported in an account by Albert Turner, a prominent local civil rights activist at the time who was present in Marion on the night of the shooting. In an interview, he notes: "then they (state troopers) took Jimmie and pinned him against the walls of the building and ... at close range they shot him in the side" (Hungry Blues, March 30, 2005).

However, Fowler's own account of the shooting, however, is markedly different. He says that when troopers arrived at the café, bottles and bricks were thrown from the upper story of the building. Fowler, the defendant in this case, noted that upon arriving at the café people started throwing bottles and bricks from the upper story of the café on the troopers standing in the street. When he entered the café, Fowler maintains he saw an elderly black woman hit a trooper with a Coke bottle and that several people attacked another trooper. Fowler alleges that as he tried to assist that trooper, someone tried to pull his gun from his holster. That someone is said to have been Jimmie Lee Jackson. While struggling with Jackson over the gun, Fowler pulled the trigger; he does not remember how many times. (Fleming, March 6, 2005). Obviously, this account tends to mitigate Fowler's culpability. In Fowler's own words, "He (Jackson) was trying to kill me and I have no doubt in my mind that, under the emotional situation at the time, that if he would have gotten complete control of my pistol that he would have killed me or shot me" (Fleming, March 6, 2005).

The night that Jackson was shot, Dr. Martin Luther King, Jr. wired Nicolas Katzenbach, who was then the Attorney General of the United States under President Johnson. King's view of the shooting was that it could have a dangerous ripple effect: "This situation can only encourage chaos and savagery in the name of law enforcement unless dealt with immediately" (Fleming, April 2005) 11). Apparently, the Federal Bureau of Investigation was knowledgeable about the case because Katzenbach informed King that the Justice Department had already launched an investigation into Jackson's shooting. Interestingly, one source notes, "Forty years later, no Justice Department official has ever questioned the trooper about the death of Jackson" (Fleming, April 2005).

After being shot by Fowler, Jackson ran from the café to the church but was beaten by law enforcement personnel along the way. He fell, was picked up and carried to a local hospital in Marion, and was then transferred to a hospital in Selma (Hungry Blues, March 30, 2005).

The Death of Jimmie Lee Jackson

Good Samaritan Hospital was directed by a small order of Roman Catholic priests, of Priests, the Edmundites, and staffed by nuns. According to one source, it was more of a clinic to which African-Americans had access in the segregated south at the time. The source also describes Jackson's condition upon arrival: "Gunshot wound of abdomen—noted hole in left side also. Laceration on back of head appears 2 inches long.... Was shot by a state trooper in riot . . ." (Fleming, May 17, 2007).

One of those who visited Jimmie Lee Jackson at the hospital was Colonel Al Lingo, head of the Alabama State Police at the time Jackson was shot. He was described in the following manner: "Four days later, Col. Lingo walked into the hospital. He was in full uniform, lightning bolts on his service cap, silver eagles on his collar, a warrant in his hand. He read aloud the state's charges against Jackson: assault and battery with intent to murder a peace officer" (Ognibene, May 2010)15).

As is to be expected, a patient in the hospital will have contact with staff members, and such was the case with Jackson. Among others who spoke with Jackson while he was at the Good Samaritan Hospital was a nurse. According to one source," legal experts suggest the prosecution's case could hinge in large part on the testimony of a former nurse at Good Samaritan who reportedly spoke with Jackson before he died. Under Alabama law, a so-called dying declaration – a statement that otherwise could be considered hearsay—may in certain conditions be admissible in court" (Fleming, May 17, 2007).

An interesting twist in the case is that the physician who attended to Jackson at Good Samaritan said he was killed by an anesthesia dosage. Dr. William Dinkins said in a 1979 interview gathered as part of the documentary film Eyes on the Prize that "In my opinion, Jimmie Lee Jackson died of an overdose of anesthesia"(Fleming, May 25, 2007). However, another doctor who had experience tending to gunshot wounds indicated later that the interview does not give a clear idea of the cause of death. The prosecuting attorney considered these details irrelevant. He was quoted as saying "If he (Jackson) wouldn't have been shot, he wouldn't have been in the hospital" (Fleming, May 25, 2007).

Dr. King and Jimmie Lee Jackson

After Jackson's death, Dr. Martin Luther King eulogized him by noting "I never will forget as I stood by his bedside a few days ago . . . how radiantly he still responded, how he mentioned the freedom movement, and how he talked about the faith that he still had in his God. Like every self-respecting Negro, Jimmy Jackson wanted to be free. . . . We must be concerned not merely about who murdered him but about the system, the way of life, the philosophy which produced the murderer. . . ."(Stanford Encyclopedia, Martin Luther King).

Dr. King also enunciated other pertinent comments when he spoke at the funeral of Jimmie Lee Jackson:

A state trooper pointed the gun, but he did not act alone. He was murdered by the brutality of every sheriff who practices lawlessness in the name of law. He was murdered by the irresponsibility of every politician, from governors on

down, who has fed his constituents the stale bread of hatred and the spoiled meat of racism.

He was murdered by the timidity of a federal government that can spend millions of dollars a day to keep troops in South Vietnam and cannot protect the rights of its own citizens seeking the right to vote. He was murdered by the indifference of every white minister of the gospel who has remained silent behind the safe security of his stained-glass windows.

And he was murdered by the cowardice of every Negro who passively accepts the evils of segregation and stands on the sidelines in the struggle for justice. (Ognibene, May 25, 2010).

The following quote aptly explains the significance of Jackson's death to the civil rights movement and may reflect an example of path dependence as well as the role of martyrs in changing society in a meaningful way:

The death of Jimmie Lee Jackson set in motion a train of events that stirred a lethargic nation and compelled once-reluctant politicians to use the power of federal law to ensure that every adult citizen could register and vote. Denied that birthright in life, Jimmie Lee Jackson by his death shamed politicians to take up a cause that many had long ignored, giving millions of his brothers and sisters, once voiceless, the right to determine who will hold office and the power to hold them to account for their deeds and misdeeds (Ognibene, May 25, 2010).

Following Jackson's death, there was talk among civil rights advocates of taking his body to the state capitol at Montgomery and reminding Governor George Wallace of the ill treatment suffered by advocates of increased voting opportunities for minorities in the state. But instead, Dr. King's organization, the Southern Christian Leadership Conference organized a march from Selma to Montgomery in response to Jackson's death. As the marchers began to cross the Edmund Pettis bridge in Selma, they were stopped by Alabama troopers using violence in a way that quickly captured the attention of the nation and the world since much of it was shown on television. The incident at the bridge is viewed by many today as a prominent motivating factor in the passage of the Voting Rights Act of 1965. It should be noted that during this period, there were also a number of other killings in the area of the march involving civil rights advocates such as Reverend Reeb and Mrs. Viola Liuzzo (King Encyclopedia).

Political Ramifications—Forty Years Later

In March of 2005, an Alabama newspaper, The Anniston Star, published a number of articles about the shooting of Jimmie Lee Jackson. Shortly thereafter, the Alabama State Legislative Black Caucus called for an investigation of the shooting. In reference to the shooting, a prominent member of the caucus, State Senator Hank Sanders, recalled recent convictions of individuals associated with older crimes against civil rights activities: "The least we can do is to seek vigorous prosecution of this case" (Anniston Star, March 31, 2005). Subsequently, the Alabama Attorney General's Office indicated that it would review the case to determine if a new investigation was warranted. In addition, Alabama Governor Bob Riley posted a reward of \$5000 for information that would lead to a conviction (Selma Times Journal,

Voting Rights Act of 1965

September 12, 2006). Governor Riley was quoted as saying, “The person responsible for this murder should be brought to justice” (Greenberg, 2006).

The Indictment

Thomas Jones, the presiding circuit judge in this case, indicated, that he had “some questions about this case because federal and state grand juries reviewed the shooting shortly after it occurred and brought no charges” (Rawls, September 6, 2009). Yet, during the forty years after the death of Jimmie Lee Jackson—a number of important changes had occurred for African-Americans in Alabama. One possible change resulting from the Voting Rights Act of 1965 was the election of African-Americans to political office. One such official was Michael Jackson, who became the district attorney in the jurisdiction in which Jackson was shot. In 2007, he was successful in securing an indictment against former Alabama State Trooper Fowler. It took the grand jury only two hours to return the indictment. Fowler was charged with first degree murder, which is an intentional act, and second degree murder, which is an unintentional act (Associated Press, May 10, 2007.)

Possible Evidence

Although many years had passed since the shooting, the prosecution believed it had enough evidence to go to trial. District Attorney Jackson was quoted as saying, “We have witnesses, we have photographs. “We have all kinds of evidence” (Fleming, October 7, 2008). Interestingly, the judge who was originally scheduled to hear the case ordered the district attorney to provide the defense a list of witnesses he planned to present at trial and provide a summary of their expected testimony. The prosecution argued that this was improper, and this view was upheld by the Alabama Supreme Court (Rawls, May 8, 2010).

Possible Defense Strategy

The defense could have employed any of a number of strategies. For example, even though Fowler admitted shooting Jackson, the defense could have argued that it was done in the line of law enforcement duty. It could also have suggested that the shooting was a form of self-defense in view of the alleged attacks on law enforcement personnel outside and inside of Mack’s café during that evening. Additionally, it could have brought into evidence a suggestion that Jackson actually died of an overdose of anesthesia at the Good Samaritan Hospital.

Importance of a Jury

Up until the passage of the Voting Act of 1965, few African-Americans served on Alabama juries. However, things have changed in the South and in Alabama, and more African-Americans today do serve on juries. This could have been an important asset to the prosecution, especially if these jury members remember either from their own personal experiences or from events related to them by elders about how Alabama law enforcement officers acted unfairly toward civil rights activists in the 1960s. Hence, it would seem that a strategy for the prosecution could have been to place as many African-Americans on the jury as possible. On the other hand, perhaps a strategy for the defense would have been to keep blacks off the jury and try to increase the number of older, conservative Caucasians who might be more sympathetic to older times in the state.

Why It Took So Long For the Case to Conclude

It took a long time for the case to conclude. The basic reason for this seems to have been objections relating to court procedure. In May of 2010, a source explained the delay:

The case has been delayed by factors ranging from an appeal of a judge's order to the sparse court schedule in rural Perry County, which only hold court a few weeks each year. In the appeal, the prosecution challenged an order that they give the defense a list of potential witnesses and their testimony. The Alabama Supreme Court ruled the judge went too far. Other motions yet to be resolved include one by the prosecution saying the judge who is white, has become Fowler's "guardian angel" and should turn the case over to a new judge. The defense is also waiting for a ruling on its claim that a fair trial is impossible in Marion, where historic markers memorialize Jackson as a martyr and a play about him extended its run last year because it drew such large audiences. (Rawls, May 8, 2010).

Delaying the trial could have been advantageous and disadvantageous to both sides. For example, the delay would have given both sides more time to prepare their cases and obtain more evidence. Yet as time passed, the witness list for each side could have been diminished. Doug Jones, a former U.S. Attorney who prosecuted Klansmen indicted in 2000 on charges involving the famous Birmingham church bombing, indicated that the biggest risk in civil rights-era cases is having witnesses die or suffer a medical problem that makes them unable to testify (Rawls, May 8, 2010). In addition, the witnesses may still feel intimidated and reluctant to testify based on past unpleasant memories or experiences involving civil rights matters.

Marion, Alabama—Some Forty Years Later

In Marion, Alabama, some forty years after the shooting of Jimmie Lee Jackson, some things have changed while others remain the same. It is still basically a poor, rural, agricultural area with a large African-American population. It differs in that today African-Americans have more involvement in local politics and more opportunities to improve their lives by using the political process. Yet, one source recognizes a need for a sense of justice and a desire to know the truth about the death of Jimmie Lee Jackson. Here are some local views on the effect of Jackson's shooting.

"Without Jimmie Lee Jackson there would not have been a Selma March."—from the owner of a funeral home

"This place here is where it all started. It was because of Jimmie Lee that it all happened. This is the cradle of the Civil Rights Movement."—from a deputy sheriff who is the cousin of Jimmie Lee Jackson

Black people all across America enjoy freedom because of Jimmie Lee. Marion lit the fire."—from the son of a prominent Civil Rights leader (Fleming, March 6, 2005).

Jackson's daughter was only four years old when her father was killed; she wonders when the gaps about her father's death will be filled. She was quoted as saying, "There's a void in my life I want to fill" and "It's crazy. I can't understand why it is taking so long" (Rawls, May 8, 2010).

The Effects of A Possible Trial

A trial, had it taken place, might have had several possible effects. For example, it would have received considerable attention on both the state and national levels, primarily because the case is related to the famous Selma March and the Voting Rights Act of 1965. It also would have caused some individuals to evaluate the progress that has been made in this country relating to furthering the civil rights of minorities. In addition, a guilty verdict against Mr. Fowler might have reinforced a view that Alabama law enforcement operated in a very negative way toward civil rights advocates in the 1960s. Of course, had he been convicted in a jury trial, Mr. Fowler could have appealed his case to a higher court, thereby delaying even further the case's conclusion. If Mr. Fowler, however, had been found innocent, then his account of the events in Marion some forty years ago could be given much more credence. Yet, Pulitzer Prize-winning author Taylor Branch, who wrote a number of books about the civil rights movement, noted that a trial was important not only for justice but for history: "Millions of people are voting today who might not be voting if not for that case" (Rawls, May 8, 2010) Whatever the result of a trial had been, the death of Jimmie Lee Jackson will go down in history as an important link to the advancement of opportunities for minorities in this country.

Major Figures in the Case

"Jimmie Lee Jackson"

Jimmie Lee Jackson is described in an interesting manner: Jimmie Lee had a wispy mustache, gentle smile, and weariness in his face that belied his 26 years. Some of it reflected the hard work he did felling trees for six dollars a day. More of it, perhaps, came from his frustration as a young black man and Army veteran whose city, county and state had denied him the right to vote.

Over a four-year period, Jimmie Lee had gone to the voting registrar's office in Marion, often accompanied by his elderly grandfather. A federal court had ordered the county to open its registration to all regardless of race, but each time Jimmie Lee and his grandfather went to the courthouse, the registrar used one stratagem or another to prevent their becoming voters. (Ognibene, May 25, 2010).

"James Bonard Fowler"

Mr. Fowler had an interesting career and indicated how the case has affected his life: Until Fowler's indictment, he was living a quiet life on a small farm near Geneva in southeast Alabama.

In 1968, he was fired from the highway patrol for beating a supervisor. He became an Army Ranger and received Silver Stars for service in Vietnam.

Fowler said he has been forced to sell farmland to pay his legal bills -- \$50,000 up front and \$1,000 a month -- and is now broke.

Every day I think they want to keep it open to wear me down until I die.
I feel like my rights have been violated. If I was black, it wouldn't be like that, and everybody knows that, . . . (Rawls, May 8, 2010).

"Tommy Jones--Possible Presiding Judge"

The presiding judge in the trial was to be Alabama Circuit Judge Tommy Jones. Such a judge has original jurisdiction over criminal felony cases in the state. However, apparently he

had questions about the case since it was reviewed by federal and state grand juries shortly after the shooting, and neither grand jury indicted Mr. Fowler. Differences of opinion about the case have caused the prosecutor to ask the judge to step aside and allow a different judge to hear the case. The judge reportedly retained an attorney (Rawls, September 6, 2009). These differences of opinion between the judge and the prosecutor were considered to be a reason for the case not reaching the trial stage.

“Michael Jackson--The Prosecutor”

District Attorney Michael Jackson had a strong interest in the killing of Jackson for a long time as noted in this published information from an Alabama newspaper:

The case has haunted Jackson since his days as an assistant prosecutor and presented itself as an opportunity to bring about some closure. This closure was needed, he says, because no one was ever prosecuted for Jimmy Lee’s death, a casualty in a people’s endeavor for human rights. The district attorney’s father, the late Claude Jackson, was elected and served as the first black chairman of the Sumter County Commission when Jackson was in high school. Jackson 42, went on to graduated from Centre College in Danville, Kentucky, and Florida State Law School in Tallahassee, Florida. He lived in Marion for a short time after law school, which is where his dream of bringing Jimmy Lee’s killer to justice began. . . . After Jackson took office in January 2005, he met with the Alabama Bureau of Investigation, and was assigned an agent, Johnny Tubbs, to assist with the investigation. Elected officials from throughout the Black Belt have lauded his effort, but not much evidence exists more than 40 years later. He said the best way to get justice is to find an eyewitness. (Selma-Times, Sept 12, 2006).

“George Beck--The Defense Attorney”

It seemed that Mr. Fowler was quite fortunate to have an experienced lawyer as indicated by the following information:

... D.A. Jackson is facing a defense attorney who understands civil rights era cases particularly well. Fowler's defense attorney George Beck was on the legal team that first sought new indictments in the 1963 Birmingham church bombings that killed four young girls. Now in private practice, Beck is taking on Fowler's case because he says justice is a two-way street.

... I think that we have to be real careful in discriminating between those acts of intentional violence - I don't want to sniper rifle or something like this - as opposed to the trooper who's trying to protect the public, who may be trying to act on orders of his supervisor. And I just don't think that every civil rights injury and killing means that something was done illegally. (Cornish, May 10, 2007).

Mr. Beck's ability as an attorney was recognized by others:

Some of Alabama’s top Democrats have recommended that President Obama appoint Fowler’s lead attorney George Beck, to the U.S. Attorney’s post in Montgomery. If that happens, Beck would withdraw from the case and Fowler would need a new lawyer (Rawls, May 8, 2010).

Mr. Beck had an interesting view of the case: “I think somebody is trying to rewrite history, and I don’t think it’s fair to this trooper,... (USA Today, May 9, 2007).

An End to The Case—A Matter Of Justice?

It took a long time, but the case against the Alabama state trooper finally ended. In November of 2010, some three years after his indictment in 2007, James Bonard Fowler, then

seventy-seven, pled guilty to shooting Jimmie Lee Jackson, thereby avoiding a trial. He was sentenced to six months in a county jail near his home and six months supervision. Fowler apologized for his actions but still claimed that the shooting was in self-defense: “I was coming over here to save lives, ... I didn’t mean to take lives. I wish I could redo it” (Morgan, November 15, 2010)

Following the guilty plea, *The Montgomery Advertiser* noted that Fowler would serve his sentence in a county jail near his home in Geneva, Alabama, for the misdemeanor conviction of second degree manslaughter rather than in Perry County, which is predominantly African-American and where Jimmie Lee Jackson is viewed as a martyr. Fowler still does not feel safe because of the number of African-Americans in jail. Yet the chief deputy for the Geneva County Jail indicated that the jail has safely housed for law enforcement officers before, and that Fowler could be housed in one of its private cells for high risk offenders or in a low risk area that is similar to a dormitory. He doubts that there will be a problem. *The Montgomery Advertiser* noted that Fowler chose to plead guilty as a result of a judge’s refusal to move his trial to a different county and because he did not believe he could get a fair trial. Fowler also indicated that he spent more than \$100,000 on his defense and sold farmland and cattle to pay his bills. He noted, “it broke me” (December 1, 2010). But was justice served upon his conviction? Perhaps it depends on who is asked. Albert Turner, Jr., an African-American county commissioner, referred to the verdict as a “slap in the face of the people of this county” (Brown, November 15, 2010).

Cordelier Billingsley, the daughter of Jimmie Lee Jackson, also expressed disappointment: “This is supposed to be closure, but there will never be closure” (Morgan, Dec. 27, 2010). Yet, Michael Jackson, the district attorney who prosecuted the case, noted: “Time was running out,... We wanted to make sure justice was done before he (Fowler) died” (Brown, November 15, 2010). The district attorney also recommended the plea to the family and indicated that he wanted Fowler to admit what he did, apologize to Jimmie Lee’s family, and spend some time behind bars. He also noted, “This is almost like a death sentence for him at his age” (Morgan, November 15, 2010). Defense attorney George Beck indicated that Fowler did not believe he could get a fair trial in Perry County and that his health is poor. He also noted, “He wants to put it behind him,” and that “It puts to rest a long chapter of civil rights history here in Perry County” (Morgan, November 15, 2010).

Even though there are differences of opinion about the outcome of the case, there are some positive aspects. For example, it has served to educate many people about the civil rights movement of the 1960s. It also reminds us of the social and political injustices of that period as well as the many sacrifices so many individuals had to make to bring more fairness for minorities into American society. Lastly, one can say that Jimmie Lee Jackson did not die in vain because his death was a major factor that led to the Voting Rights Act of 1965.

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A SURVEY OF U.S. LAWS ON HUMAN TRAFFICKING

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ABSTRACT

Contemporary slavery, or the more palatable term, human trafficking, is garnering increased media coverage and the predictable (based on media reports) subsequent legislative (re)action. The problems related to human trafficking are serious and legislation is diverse. This essay considers whether current laws regarding human trafficking are effective, consistent, or even relevant. Like governments worldwide, America's federal, state and local governments are trying to reduce the harm caused by trafficking in persons. This essay will be a comprehensive overview of state, federal and U.S. territorial legislation related to human trafficking.

INTRODUCTION

In the United States (U.S.) a historical tradition for the past 150 years has been that everyone has equal protection under the law. In fact, this consideration is even extended to visitors and aliens, who are afforded the same rights as documented, legal citizens. However, while the U.S. values freedom and equality, not everyone receives equitable legal treatment. This is particularly true for those brought into the country against their will, under false pretenses or illegally.

People are trafficked and exploited around the world daily. Slavery has a long, tragic history. The United States, despite its emphasis on individual freedom, has a sorted history. In the U.S. slavery began in the early 17th century and legally persisted until it was abolished in 1865. Slavery, or to use the more politically correct and less offensive term "human trafficking," still persists. No longer an above board, openly traded commodity, humans today are trafficked clandestinely and illegally. The early slave traders were motivated by profit. While the profit motive persists, the victims of contemporary human trafficking are often willing, if unknowing, participants due to dire economic circumstance (whereas previously, slaves were captured and transported against their will).

Destitute individuals and families in third world and developing countries are often forced, tricked or coerced into involuntary servitude (Trafficking Victims Protection, 2009). Exploiters make a profit off these desperate people by promising them jobs and prosperity in the United States and other wealthy nations. Other victims are simply kidnapped. Traffickers have been known to use any means available, including force, fraud, and coercion, to exploit and profit from the forced labor and servitude of men, women, and children. The more common destinations are brothels, factories, agriculture, fishing, and mining facilities (Sigmon, 2008).

Human Traffickin

Especially disheartening is the fact that some parents that give their children to these traffickers hoping that their children will have a chance at a better life

These often naïve people are coerced and/or tricked into servitude because they trust the traffickers. Sadly, all too often the traffickers are friends, family members, or trusted acquaintances. Some former victims have even turned to being recruiters. These victims of human trafficking are told that all they have to do is work to pay the debt associated with transportation. This debt to be repaid with work is known as debt bondage. Historically, this debt bondage was known as indentured servitude.

The reality for victims is that the payback structure is such that the debt can never be repaid. The exploiters charge an initial fee, tack on high interest rates, and then charge exorbitant rates for food and housing. Ultimately, there is little or no money remaining for debt bondage repayment. The victims are told that they are illegal aliens in the United States, that the authorities do not care about them, and that should they turn to law enforcement for help, they will simply be arrested. Because of this manipulation on the part of the exploiters, many cases of human trafficking go unnoticed by government officials. The trafficking victims view government authority figures as threats rather than protectors. The reality is that U.S. law at the federal level and in most states and territories often does provide relief for victims and outlaws trafficking. This essay reviews those laws.

Amendment 13 of the United States Constitution abolished slavery in the United States. International law also forbids the practice:

During the 1900s, government representatives acting under the auspices of the League of Nations and the United Nations crafted international instruments to define and combat slavery's newly recognized forms- including white slavery, forced labor, debt bondage, the facilitation of child prostitution (or any commercial sex act with a child), forced prostitution or commercial sex act of an adult, and sexual slavery- and asserted that these forms of exploitation denied people their most basic human rights and that no one shall be held in slavery or servitude (Sigmon, 2008).

More contemporary legislative response in the United States to human trafficking should have been strengthened by the enactment of the Trafficking Victims Protection Act of 2000 (TVPA). This act was reauthorized and amended in 2003 and again in 2005 with the Trafficking Victims Protection Reauthorization Act of 2005 (TVPRA 2005). This act outlines a victim-centered approach to trafficking. It includes trafficking prevention programs, protection and assistance for victims, and strengthens criminal statutes for the prosecution of traffickers and increases their punishments (Sigmon, 2008). The TVPRA distinguishes between voluntary criminal behavior and forced criminal behavior. If the act were applied as enacted and intended, victims would be treated not as criminals, but rather as victims. The legislation provides access to assistance and benefits, including immigration relief that permits victims who cooperate with law enforcement to remain temporarily in the United States; later they may receive permanent resident status. There is now also a victim of human trafficking hotline provided by the Department of Justice (DOJ).

In the United States all fifty states have the prerogative to draft their own legislation aimed at traffickers. The Tenth Amendment in the Bill of Rights delegates' legislative power to the individual states; therefore there are variations among the states in their statutes dealing with human trafficking.

This essay will provide a comprehensive overview of state, federal and U.S. territorial laws on human trafficking. In order to help clarify the differences and similarities among the state laws, the states are grouped according to the 11 Federal Circuit Court districts to which they are assigned.

STATE LEGISLATION

Some states do not have any specific criminal statutes for human trafficking. Also, some states use statutes such as their kidnapping statute to define their human trafficking laws. A description of state and territorial laws, by district, follows.

First Circuit:

The first circuit is comprised of Maine, New Hampshire, Massachusetts, Rhode Island and Puerto Rico. As of March 19, 2009, Maine did not have a statute stating that the crime of human trafficking is illegal. They do, however, have a Victim's Compensation Fund that provides compensation for expenses resulting from personal injury as a direct result of the crime; covered offenses include sexual assault, kidnapping and criminal restraint, and sexual exploitation of a minor. The personal injuries that may be brought up in the civil proceeding against the trafficker include medical and mentally related expenses, as well as psychological and mental health counseling expenses (Victims Compensation Fund, 2009).

Maine also has a statute that deals with the civil remedies for human trafficking. While the traffickers will be investigated and prosecuted for the offense of human trafficking, the trafficking victim may also bring civil action against the trafficker. The victim has a period of ten years from the date the victim was released from the trafficking situation in which to sue the trafficker for actual damages, compensatory damages, injunctive relief, and any other form of appropriate relief. The statute of limitations of ten years does not apply if threats by the defendant cause duress to the plaintiff or a delay of filing. If the victim is deceased, a legal guardian or family member may sue on behalf of the victim (Civil Remedies for Human Trafficking, 2009).

New Hampshire does not have a human trafficking statute. The statute that most resembles a human trafficking statute is their kidnapping statute. The only similarity between the kidnapping statute and human trafficking statutes is that the kidnapping statute includes confinement under the control of another and avoiding apprehension by a law enforcement official (Interference with Freedom, 2009).

Massachusetts has no statutes related to human trafficking or debt bondage. While there are laws involving sexual abuse and child neglect, the forced labor or sale of persons is not covered under laws of the Commonwealth of Massachusetts. Like Massachusetts, Rhode Island has no anti-human trafficking laws, only a rather vague kidnapping statute that is almost identical to that of New Hampshire (Kidnapping, 2009).

Puerto Rico's constitution is the only legislation in that territory that outlaws slavery and involuntary servitude. Under Article II of their constitution, slavery and involuntary servitude are illegal unless imposed as a sentence for a criminal conviction. Any persons found guilty of slavery or involuntary servitude will have their civil rights suspended and right to vote taken away while imprisoned for this crime (Constitution of the Commonwealth of Puerto Rico Article II. Bill of Rights, 2005).

Aside from Maine, no other states in the first circuit of the United States have any programs to help victims of human trafficking. Maine therefore appears to be more proactive

with assisting victims compared to the other states in this circuit. Kidnapping statutes in this circuit are quite vague, with little ties to the issues of human trafficking and involuntary servitude. Conceivably these states may recognize the need for better-articulated anti-trafficking legislation.

Table 1: 1st Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Maine	N/A	N/A	Actual/Civil	Yes	Human Trafficking
New Hampshire	A	N/A	N/A	No	Kidnapping
Rhode Island	A Loss of civil rights	20 years	N/A	No	Kidnapping
Puerto Rico	Varies	N/A		Yes	Slavery
Massachusetts	N/A	N/A	N/A	No	Sex Abuse

Second Circuit:

The second circuit of the United States includes Vermont, New York and Connecticut. While Vermont does not have an anti-human trafficking statute, it does have a more in-depth kidnapping statute than the states in the first circuit. Vermont's kidnapping statute defines that a person commits the crime of kidnapping if the person knowingly restrains another to facilitate the commission of another crime. This statute is more fit as an anti-human trafficking statute because traffickers do, in fact, often use victims to commit crimes such as prostitution, drug smuggling, and child labor (Kidnapping, 2009).

In New York, a person is a victim of human trafficking if that person is a victim of sex or labor trafficking. As of April 7, 2009, there are services for victims of human trafficking under New York Social Services Law. This service may be given to "pre-certified victims of human trafficking" (Services for Victims of Human Trafficking, 2009). Non-government organizations (NGO's) are funded in order to provide services to victims. These services may include, but are not limited to, case management, health care, mental health counseling, drug addiction treatment, translation services, and job training. These services also assist some victims with establishing permanent residence in New York or another part of the United States (Services for Victims of Human Trafficking, 2009).

The services for victims are part of a New York interagency task force for combating the issue of human trafficking in their state. The task force collects and analyzes data on the nature of human trafficking in the state; provides federal, state, and local services to victims; consults with governmental and nongovernmental organizations to strengthen state and local efforts on combating the issues associated with human trafficking (prevention, protection, and prosecution); and increases public awareness (Services for Victims of Human Trafficking, 2009). Connecticut does not have a specific anti-human trafficking statute or any laws dealing with debt bondage. The only Connecticut law that relates to human trafficking laws is their offenses against abduction of adolescents. This statute includes kidnapping but applies only to children aged 16 years and under (Offenses Against the Person, 2008).

The states in the second circuit have more legislation in the area of human trafficking laws than do the states in the first circuit. Vermont and Connecticut's kidnapping statutes are the closest thing those states have to an anti-human trafficking law. While Vermont's kidnapping statute addresses the general issue of human trafficking, Connecticut's focuses more on the issue of trafficked children. Out of the three states in this circuit, New York by far provides the best protection for victims of trafficking. New York also goes further with the use of the task force, to combat the issue of human trafficking.

Table 2: 2nd Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Vermont	N/A	Max 30 years	\$50,000	No	Kidnapping Victim/ Human
New York	N/A	N/A	N/A	Yes	Trafficking
Connecticut	D	Repealed	N/A	No	Kidnapping

Third Circuit:

The third circuit of the United States includes Pennsylvania, New Jersey, Delaware, and the Virgin Islands. A subsection of Pennsylvania's kidnapping statute describes unlawful restraint. A person may be charged for the crime of unlawful restraint if he/she holds another in a condition of involuntary servitude. This crime is a misdemeanor in the first degree. If the victim is under the age of 18, the offense increases to a felony in the second degree (Kidnapping, 2009). Aside from this unlawful restraint statute, Pennsylvania has no specific anti-human trafficking legislation.

New Jersey, on the other hand, does have a human trafficking statute. Under New Jersey law, a person commits the crime of human trafficking if she knowingly holds, recruits, lures, entices, harbors, transports, provides or obtains, another to engage in a sexual activity. This crime is punishable by 20 years imprisonment without parole, or a sentence of 20 years to life imprisonment (Kidnapping; Coercion, 2009). This statute is quite comprehensive in that it makes every step of the process of trafficking in persons a criminal offense. It also allows for restitution to be paid to the victim. The trafficker may end up paying for the work the victim did but was never paid for, and for anything that resulted in personal injury to the victim.

Delaware, like some other states, uses its kidnapping statute to prosecute traffickers. The connection between anti-human trafficking legislation and Delaware's kidnapping statute are the terminology "unlawful restraint". Victims of human trafficking are being held against their will working to pay the debt bondage they owe to their traffickers (Kidnapping and Related Offenses, 2009). Finally, a subsection of Delaware's kidnapping statute deals with trafficking of persons and involuntary servitude. Of the states discussed so far, this is the first state that specifically defines human trafficking and involuntary servitude under its kidnapping statute. A person may be charged for the crime of trafficking of persons and involuntary servitude if that person forces another into a commercial sexual activity, forced labor or services, or any sexually explicit performance (Kidnapping and Related Offenses, 2009).

Not much could be found on the human trafficking legislation of the Virgin Islands. A search conducted through LexisNexis Academic, found only one mention of human trafficking. Title 11 of the Virgin Islands Code, section 3A, provides for a response to human trafficking victims (Trafficking of Persons, 2009).

Overall, human trafficking laws in the third circuit are much stronger than in the first and second circuit. While Pennsylvania has some legislation in place under its kidnapping statute, Delaware's kidnapping statute appears superior because it is much more specific about what is considered a human trafficking crime. This is the first state discussed so far with a kidnapping statute that comprehensively covers human trafficking crimes. It makes involuntary servitude and trafficking crimes in themselves, not just kidnapping. New Jersey has a specific human trafficking statute. Like Delaware's kidnapping statute, it defines the wide range of crimes under human trafficking. In New Jersey, the crime is punishable by 20 years to life in prison. Also, traffickers must pay restitution to their victims. Not much data could be located related to human trafficking as a crime in the Virgin Islands.

Table 3: 3rd Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Pennsylvania	1	N/A 20 years - life	N/A Lost wages	No Yes Yes (under kidnapping)	Kidnapping Kidnapping/Coercion Kidnapping Trafficking of Persons
New Jersey	1				
Delaware	A-F	N/A	wages		
Virgin Islands	N/A	N/A	N/A	Yes	

Fourth Circuit:

The fourth circuit of the United States is comprised of Maryland, Washington D.C., West Virginia, Virginia, North Carolina, and South Carolina. Maryland's human trafficking statute is worded quite differently from other human trafficking legislation discussed thus far. Their human trafficking statute is covered under prostitution and related crimes. A person is guilty of human trafficking if that person knowingly takes or causes another to be taken to a place of prostitution, harbors a person for prostitution, or unlawfully detains another with intent to use force, threat, or persuasion to perform a sexual act or contact. Minors are also covered under this statute (Prostitution and Related Offenses, 2009).

There is currently no legislation in place in Washington, D.C. on human trafficking. There is also no kidnapping statute that covers human trafficking crimes, nor is there legislation for the purpose of prosecuting traffickers or protecting victims.

As of July 7, 2009, there were also no anti-human trafficking statutes in West Virginia. That state's abduction of persons and kidnapping statute comes closest to a human trafficking statute: section A states that it is unlawful to take a child under the age of sixteen away for the purpose of prostitution (Crimes Against the Person, 2009).

In Virginia, the Commission on the Prevention of Human Trafficking was established (July, 1, 2009) in order to implement a state plan of human trafficking prevention. The commission was to address all aspects of human trafficking, from recruitment and abduction to receipt of persons (Commission on the Prevention of Human Trafficking, 2009).

North Carolina has a well-defined human trafficking statute. A person can be charged with the crime of human trafficking if that person knowingly recruits, entices, harbors, transports, provides, or obtains by any means another person with the intent that the other person be held in involuntary servitude or sexual servitude. Victims, whether legal resident's of North Carolina or not, are eligible for public benefits and services (Offenses Against the Person, 2009).

Like some other states, South Carolina has an addendum about trafficking in persons under its kidnapping statute. Any person who knowingly subjects another person to forced labor or services or does anything in the way of harboring, transporting, or enticing a person into forced labor or services is guilty of a felony and may be imprisoned for not more than fifteen years. A person who aids, attempts, or conspires to do any of part of the previously stated crimes is guilty of the same offense and may be punished the same way (Kidnapping, 2008).

Table 4: 4th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Maryland	Felony	25 year	15,000	Yes	Human Trafficking
West Virginia	Felony	10 years	N/A	Yes	Kidnapping
Virginia	Rule as expired	N/A	N/A	Yes	Kidnapping
North Carolina	F - Adult/C- Child	N/A	Public benefits	Yes	Human Trafficking
South Carolina	Felony	15 years	N/A	Yes	Kidnapping

The states in the fourth circuit have varying concept's regarding human trafficking. Maryland's legislation focuses on prostitution as the main problem associated with human trafficking. Surprisingly, Washington, D.C. has no specific legislation on human trafficking, nor is it included in that district's kidnapping statute. West Virginia, like Maryland, focuses on the problem of prostitution in relation to human trafficking. West Virginia's law states that kidnapping a child under sixteen years of age for the purpose of prostitution is illegal. Virginia has made an attempt to combat the problem by enacting the Commission on the Prevention of Human Trafficking. North Carolina's statute on human trafficking focuses on more than just prostitution; it defines sexual servitude and involuntary servitude as they relate to human trafficking. South Carolina's kidnapping statute describes the penalties for forced labor and services and trafficking people.

Fifth Circuit:

Mississippi, Louisiana, and Texas make up the Fifth Circuit of the United States. In

Human Trafficking

Mississippi, the Anti-Human Trafficking Act prohibits conduct associated with human trafficking. Under this act, a person who recruits, harbors, or transports another for the purpose of forced labor or services will be guilty of human trafficking. The person who subjects another to forced labor or services will be charged with procuring involuntary servitude. The person found guilty of either of these crimes may be imprisoned for no more than twenty years. This act further states that forcing a minor to engage in commercial sexual activity or sexually-explicit performances, or to be part of the production of sexually oriented material is punishable by no more than thirty years imprisonment (Anti- Human Trafficking Act, 2008).

Under Louisiana's human trafficking statute, the penalties for trafficking vary based on what the trafficker subjects the victim to. For example, if the trafficker forces a person into providing services or labor, that trafficker may be imprisoned and forced to do hard labor for no more than ten years. However, if the trafficker forces the victim into services that include commercial sexual activity or any sexual conduct, that trafficker may be imprisoned and forced into hard labor for no more than twenty years. The person who forces a minor (a person under the age of eighteen) into any form of forced labor or service will be imprisoned for no less than five years and no more than twenty-five years (Kidnapping and False Imprisonment, 2009).

Texas has no human trafficking legislation. It does, however, support victims of human trafficking under its Sexual Assault Program Fund, which provides grants to support programs that assist victims (Sexual Assault Program Fund, 2007).

Mississippi is the first state discussed so far that has an Act dedicated to the problem of human trafficking. Of the states in the fifth circuit, only Texas has no anti-human trafficking legislation. It does however provide support to victims of human trafficking. Louisiana's statute on human trafficking provides different penalties for certain aspects of the crime of human trafficking. It is also the first state discussed to force violators into doing physical labor.

Table 5: 5th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Mississippi	N/A	30 years	N/A	Yes	Human Trafficking
Louisiana	N/A	25 years	25,000	Yes	Human Trafficking
Texas	N/A	N/A	N/A	No	N/A

Sixth Circuit:

The sixth circuit of the United States is made up of Michigan, Ohio, Kentucky, and Tennessee. Michigan's statute for human trafficking is the most detailed compared to the states and territories discussed to this point. There are sections that describe the different aspects of forced labor and service and the penalties associated with committing that particular crime. Crimes against minors in regards to human trafficking are also included (Human Trafficking, 2009).

In Ohio, human trafficking is defined as a scheme or plan for compelling a person to engage in sexual activity for hire, or do anything obscene, sexually oriented, or nudity oriented. The penalties for human trafficking are based on the degree of the felony (Penalties and Sentencing in General, 2009).

Kentucky has a human trafficking statute. A person is guilty of a class C felony if found guilty of human trafficking and a class B felony if the victim was under the age of eighteen. Human trafficking is defined as compelling a person to engage in forced labor or services or commercial sexual activity through coercion. If the victim is under the age of eighteen, coercion is not necessary (Prostitution Offenses, 2009).

Tennessee has a statute that defines human trafficking as trafficking for forced labor or services or trafficking for sexual servitude. This state also has the Tennessee Human Trafficking Act of 2007, which describes in great detail every aspect of human trafficking (Kidnapping and False Imprisonment, 2009).

The four states in the sixth circuit have human trafficking legislation. Michigan describes all aspects of human trafficking and the penalties associated with it. Ohio's statute deals with human trafficking only with regards to sex. Coercion plays a large role in Kentucky's human trafficking statute. Coercion is necessary to charge a person with human trafficking of an adult but not of a minor. Tennessee did its part in combating human trafficking by enacting the Tennessee Human Trafficking Act of 2007.

Table 6: 6th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Michigan	N/A	10 years	N/A	Yes	Human Trafficking
Ohio	N/A	N/A	N/A	Yes	Human Trafficking
Kentucky	C	N/A	N/A	Yes	Human Trafficking
Tennessee	B	N/A	Gross Income	Yes	Kidnapping

Seventh Circuit:

Wisconsin, Illinois, and Indiana make up the seventh circuit of the United States. Wisconsin's human trafficking statute is found under chapter 940, Crimes Against Life and Bodily Security. This statute defines human trafficking as a class D felony if the trafficking is done for the purpose of commercial sex acts or labor or services and is done by fraud, debt bondage, controlling an individual by using controlled substances, or destroying, confiscating, or concealing identification documents of another. Wisconsin's statute is the first discussed thus far that defines debt bondage (Crimes Against Life and Bodily Security, 2008).

Illinois' human trafficking statute covers most involuntary servitude. This state's statute is constructed like those of other states with actual anti-human trafficking legislation. When someone is found to be a trafficker of persons, the money or property obtained by the trafficker by using the victims must be forfeited (Trafficking of Persons and Involuntary Servitude, 2009).

While Indiana does have a human trafficking statute, it is worded quite differently from those of other states. It says that promoting human trafficking and merely stating (intent) you wish to pay for a person, either adult or minor (less than 18 years of age) is a human trafficking offense. Unlike some states, Indiana focuses only on the prostitution aspect of sex crimes. They

Human Traffickin

also make reference to forced labor and involuntary servitude in their statute (Human and Sex Trafficking, 2009).

The three states in the seventh circuit have actual human trafficking statutes. Wisconsin was the first of the states discussed to make an actual reference to debt bondage in the verbiage of their statute. The human trafficking laws in Illinois are very weak, noting only that money or property obtained by the trafficker by use of the victim must be seized and forfeited. Indiana is the only state discussed so far that makes the promoting of human trafficking or the mere statement of wishing to purchase a person a criminal offense.

Table 7: 7th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Wisconsin	D	N/A	N/A	Yes	Human Trafficking
Illinois	Various Degrees	Max.	Lost wages	Yes	Human Trafficking
Indiana	A, B and C	N/A	N/A	Yes	Human Trafficking

Eighth Circuit:

The eighth circuit of the United States includes Minnesota, North Dakota, South Dakota, Nebraska, Iowa, Missouri, and Arkansas. Minnesota has created The Human Trafficking Task Force through their Department of Public Safety (DPS). They also have a toll free hotline that victims may call to report an incident. This hotline operates twenty-four hours a day, 365 days a year. Its purpose is to give legal assistance to domestic and international victims (Human Trafficking Task Force, 2008).

North Dakota is attempting to join the other states that have anti-human trafficking legislation. A bill is currently in their senate to outlaw the crime. It will be linked to their current racketeering statute. This bill also makes a reference to debt bondage. Under their version of the statute, a person is guilty of human trafficking if he or she benefits financially or receives anything of value as a result of knowing and/or participating in human trafficking. If enacted this bill will cover both labor and sex trafficking (Senate Bill 2209, 2009).

North Dakota's neighbor, South Dakota, has no legislation related to the issue of human trafficking. The only connection that could be made is in their kidnapping statute. Even less inclusive than those of other states, this statute says that a person commits the offense of kidnapping if that person confines another for the purpose of using that person to committing any felony. In many cases, the victims of human trafficking are used to facilitate another crime, some of these being felonies. A trafficker could then be charged with kidnapping in South Dakota (Kidnapping, 2009).

Nebraska, on the other hand, has a well-defined human trafficking statute. It places a large emphasis on the use of threats in forcing a person to do labor or a service. Surprisingly, it fails to mention the use of adults in commercial sexual activity, referencing only minors in this part of the statute. However it includes both adults and minors in describing forced labor and service (Offenses Related to Morals, 2009).

Iowa also has a human trafficking statute. This state's statute is very straight forward describing different aspects of human trafficking: financial gain, soliciting service, threatening

the victim, and the penalties for various crimes. All crimes under this statute are felonies. Crimes against victims under the age of eighteen are upgraded to the next highest felony. A difference should be noted in comparison to other state legislation on this matter: Iowa's statute makes it illegal to use a victim to abuse the legal process. This state recognizes both sex trafficking and labor trafficking as crimes (Human Trafficking, 2008). Victims of human trafficking in Iowa are afforded the same rights as victims of other crimes, including compensation. Their rights do not depend on their immigration status (Victims of Domestic Abuse, 2008).

Table 8: 8th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Minnesota	Task Force	N/A	N/A	N/A	N/A Human
North Dakota	A, AA	1 year	N/A	Yes	Trafficking
South Dakota	1st, B, C	N/A	N/A	Yes	Kidnapping Human
Nebraska	II-IV	N/A	N/A	Yes	Trafficking Human
Iowa	B, C, D	N/A	Lost wages	Yes	Trafficking Human
Missouri	D	N/A	N/A	Yes	Trafficking
Arkansas	C	N/A	N/A	No	Kidnapping

Unlike states discussed previously, Missouri's statute makes contributing to human trafficking a felony offense. A person may violate this statute if he or she misuses documentation in order to restrict the ability of the victim to leave, forcing the victim to do labor or services. This use of control by a trafficker does not carry a penalty of registering as a sex offender if found guilty (Sexual Offenses, 2009).

Arkansas does not have a human trafficking statute. One could argue, though, that their false imprisonment statute may be fitting for some human trafficking crimes. Under this statute, if a person knowingly restrains another so that the victim's personal liberties are violated or his or her health is in jeopardy due to substantial risk of serious physical injury, that person is guilty of a felony (Kidnapping and Related Offenses, 2009).

Minnesota and New York both have human trafficking task forces. Minnesota also has a toll free hotline for victims to report incidents. Four out of the seven states in the eighth circuit have actual human trafficking statutes: North Dakota, Nebraska, Iowa, Missouri, and Arkansas. Perhaps North Dakota will join other states in combating human trafficking with the passage of their senate bill. A trafficker may be charged with kidnapping in South Dakota if the victim is confined for the purpose of committing a felony. While Nebraska does have anti-human trafficking legislation, it fails to include adults in the section that involves sex crimes. Iowa makes it a human trafficking crime to abuse or threaten to abuse a legal process. While of the states in the eighth circuit have identified human trafficking as an issue they differ in their ways of combating it.

Ninth Circuit:

Washington, Oregon, Montana, Idaho, California, Nevada, Arizona, Alaska, Guam, Hawaii, and Micro Polynesia are the states and territories that make up the ninth circuit. Washington has focused most of its efforts on the victims of human trafficking. The state enacted a task force centered on assisting victims. Protocols were put into place making state agencies give aid to victims in the form of health care, employment, and a place to reside. Volunteers will be utilized by the task force (Victims of Crimes- Compensation, Assistance, 2009).

A person violates Oregon's trafficking in persons statute if that person recruits, harbors, or obtains a person for the purpose of subjecting that person to involuntary servitude (Offenses Against Persons Kidnapping and Related Offenses, 2007). Benefiting financially from the victim also violates this statute. Finally, the victim may also sue the trafficker for actual damages in a civil proceeding in Oregon (Actions and Suits in Particular Cases, 2007).

Montana has no legislation in place to combat human trafficking. Its kidnapping statute does not identify human trafficking or the taking of individuals for forced labor or sex. Therefore, a connection could not be made between a kidnapping statute and human trafficking.

In Idaho, human trafficking is defined as sex trafficking in which a commercial sex act is induced by force or the recruitment, harboring, or obtaining of a person, by force, for the purpose of forced labor or service (Human Trafficking, 2009). Victims are also protected in this state (Human Trafficking Victim Protection, 2009). Idaho has made an effort to focus on the crime of human trafficking and the protection of trafficking victims.

While California does have a human trafficking statute, it has a more victim-centered approach to combating the issue. A person is guilty of the crime of human trafficking in the state of California if that person deprives another of his or her liberty with intent to commit a felony, or uses the victim for forced labor or services (False Imprisonment, 2009). Victims may bring civil action against their trafficker and may be able to obtain up to three times the amount of the actual damage done to them. Victims must bring the civil action within five years of being freed from the trafficking situation (Personal Rights, 2009).

Human trafficking is not a crime in Nevada. There is no legislation in place outlawing this crime as it is defined by most other states. Nor could kidnapping statutes be found with any connection to the issue. This state focuses more on capturing people who are paid to transport people across the border illegally into the United States (Crimes Against the Person, Trafficking in Persons, 2009).

Arizona does have anti-human trafficking legislation. In this state it is illegal to traffic an individual with knowledge that that individual will be subjected to forced labor. It is also illegal to benefit financially by using the victim. This offense is a felony in Arizona (Kidnapping and Related Offenses, 2008).

Alaska has classified the crime of human trafficking by degrees. Human trafficking in the first degree is obtaining a person and forcing him or her to engage in sexual conduct, adult entertainment, or labor (Kidnapping, Custodial Interference, and Human Trafficking, 2009). If a person benefits financially from the forced labor of another, that person is guilty of human trafficking in the second degree (Kidnapping, Custodial Interference, and Human Trafficking, 2009). The use of minors in human trafficking crimes is not mentioned in this statute.

No information could be found relating to human trafficking in Guam and Micro Polynesia's statutes. Also, Hawaii has neither human trafficking legislation nor any kidnapping statutes that relate to human trafficking. The only states, out of the eleven states and territories

that make up the ninth circuit, with human trafficking statutes are Oregon, Idaho, California, Arizona, and Alaska. Nevada's focus is not human trafficking in the victim sense but human trafficking in the illegal alien trafficking sense. Once Washington enacts its task force, it and California will both have a victim-centered approach to human trafficking.

Table 9: 9th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Washington State	N/A	N/A	N/A	Yes	Human Trafficking
Oregon	B	N/A	N/A	Yes	Human Trafficking
Idaho	N/A	25 years	N/A	Yes	Human Trafficking
California	Felony	3-8 years	10,000 and up	Yes	Human Trafficking
Nevada	B	1-10 years	N/A	No	N/A
Arizona	Class 2	N/A	N/A	Yes	Kidnapping
Alaska	B	N/A	N/A	Yes	Human Trafficking
Montana	N/A	N/A	N/A	No	N/A

Tenth Circuit:

The tenth circuit of the United States is comprised of Wyoming, Utah, Colorado, New Mexico, Kansas, and Oklahoma. Wyoming has no statute for human trafficking. A search was done on related topics: kidnapping, forced labor, and trafficking in persons. None of these searches yielded any results. Nor does Wyoming appear to be enacting human trafficking legislation.

Utah, on the other hand, does have a human trafficking statute. This state's statute is defined like those of most other states, including both labor and sex trafficking. It, too, makes it illegal to abuse legal processes. This statute defines which acts constitute forced labor and forced sexual exploitation. Debt bondage is referenced in this statute as well (Offenses Against the Person: Kidnapping, Trafficking, and Smuggling, 2009). Utah is the first state to define aggravated human trafficking under its statute. A person commits the crime of aggravated human trafficking if that person, while in the course of committing a human trafficking offense, causes great bodily harm or death; commits rape, forcible sodomy, sodomy of a child, aggravated sexual abuse and/or assault, or if the person involves more than ten victims in a single episode for more than 180 consecutive days against their will (Offenses Against the Person: Kidnapping, Trafficking, and Smuggling, 2009).

Table 10: 10th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Utah	1st	N/A	N/A	Yes	Human Trafficking
Colorado	Task Force	N/A	N/A	No	N/A
New Mexico	1st - 3rd Degree	N/A	Gross Income	Yes	Human Trafficking
Kansas	2	N/A	N/A	Yes	Human Trafficking
Oklahoma	Felony	10 years	20,000	Yes	Human Trafficking
Wyoming	N/A	N/A	N/A	No	Kidnapping

While Colorado does not have any anti-human trafficking legislation, it does have an interagency task force. The task force gathers data on human trafficking incidents in the state. It also protects victims while prosecuting traffickers. The victims are entitled to housing, health care, education, legal assistance, and job training (Interagency Task Force on Trafficking in Persons, 2008).

New Mexico's human trafficking statute mirrors that of many other states. One notable difference is the degree of felony associated with minors of different ages. Trafficking victims under the age of sixteen is a second-degree felony, while, trafficking a victim under the age of thirteen is a first-degree felony. Also, victims cannot be charged with being an accessory to the crime. Traffickers, if found guilty, must make restitution to their victims for whatever labor or service they were coerced into doing (Human Trafficking, 2009).

Kansas also has a human trafficking statute. It does not go into great detail about what constitutes forced labor. This statute does not include sex trafficking of adults as a crime. It does, however, claim that benefitting financially from the forced labor or services of the victim is illegal (Crimes Against Persons, 2008). It, too, has made aggravated trafficking a different offense, though it is defined differently than in Utah. It involves the use of minors for forced labor and service and/or sexual gratification (Crimes Against Persons, 2008).

Oklahoma is the first state discussed thus far with a statute that specifically states that human trafficking is the extreme exploitation and denial of freedom or liberty of an individual by another. This state's statute recognizes both forced labor and forced sexual exploitation. The verbiage of what violates this statute is much the same as in any other state with a human trafficking statute. Crimes against victims under the age of fourteen carry a higher penalty, as is true in most other states (Kidnapping, 2009). Oklahoma also has guidelines for the treatment of victims. Victims will not be penalized or jailed for their involvement in the crime. They are given prompt medical attention, shelter, and food. In addition, they will receive legal advisement, will be informed of their rights, and may file civil action against their traffickers (Kidnapping, 2009).

Four of the six states that make up the tenth circuit have anti-human trafficking legislation. Wyoming has no specific legislation designed to combat the issue of human

trafficking. Colorado has a task force in place to prosecute traffickers and protect victims. New Mexico has classified their human trafficking crimes as different degrees of felonies. Kansas and Utah have two different offenses for human trafficking: human trafficking and aggravated trafficking. Oklahoma recognizes the infringement of personal rights that takes place in the crime of human trafficking and also gives a great deal of help to victims.

Eleventh Circuit:

Alabama, Georgia, and Florida make up the states in the eleventh circuit of the United States. No legislation could be found in Alabama's code relating to human trafficking. All that could be found was the prohibition of slavery under the state's constitution. This is not particularly noteworthy since virtually all states outlaw slavery in their constitutions.

Georgia does not have a human trafficking statute. The only mention of any aspect of human trafficking, involuntary servitude is made in Georgia's state constitution. Involuntary servitude and slavery are outlawed in Georgia. No connections however, could be made between their kidnapping statute and human trafficking.

Table 11: 11th Circuit statutes and penalty related to human trafficking.

State	Level of Felony	Sentence	Restitution	Actual Statute	Offense
Alabama	N/A	N/A	N/A	No	Involuntary Servitude
Georgia	N/A	N/A	N/A	No	Involuntary Servitude
Florida	2	N/A	Yes	Yes	Human Trafficking

The state of Florida has anti-human trafficking legislation. Florida recognizes that many incidents of human trafficking occur within its boundaries. In this state, human trafficking may be defined as transporting, recruiting, harboring, or obtaining another person for transport (Kidnapping; False Imprisonment; Luring or Enticing a Child; Custody Offenses, 2009). Florida also has services available to assist human trafficking victims. Victims have access to state-funded medical and mental health care, housing assistance, and food while the federal government is processing them. In order to partake of these services, a sworn statement is all that is required by the victim as long as the statement includes at least one item of physical evidence. Florida also tries to bring awareness to the problem of human trafficking through these services (Social and Economic Assistance, 2009).

Florida is the only state in the eleventh circuit to have anti-human trafficking legislation. This state recognizes the crime and issue of human trafficking. It also helps victims by allowing them to take part in state-funded services. Alabama and Georgia, surprisingly, had absolutely no legislation outlawing human trafficking. The only mention of involuntary servitude, a related crime to human trafficking, was in both of these states' constitutions.

Federal Statute:

The federal government has a well-designed human trafficking statute. It recognizes forced labor and services in much the same way that the states do, with increased penalties if a death or aggravated sexual abuse occurs (Peonage, Slavery, and Trafficking in Persons, 2009). Sex trafficking of children is covered under a separate statute. The verbiage of this statute is similar to that of most states. However, because the states have the power to regulate the issue of human trafficking within their borders, the federal statute applies only to interstate or foreign commerce, maritime and territorial jurisdictions of the United States (Peonage, Slavery, and Trafficking in Persons, 2009).

In summary, of the fifty states and four U.S. territories, only twenty-three currently have anti-human trafficking legislation. Human trafficking has most certainly been identified as an issue. However, the degree to which the individual states have chosen to combat the issue through specific legislation is variable; some have drafted no specific legislation and others have extensive legislation addressing both traffickers and victims.

CONCLUSION

Legislation on human trafficking presents problems and issues not unlike those associated with border protection and illegal immigration. The two issues are linked from a law enforcement perspective, and legislation should also acknowledge and simultaneously address the overlapping and related problems. A fundamental and critical question is whether the problems related to human trafficking are best addressed at the local, state or federal levels, or by all three levels of government? Furthermore, with the few notable exceptions discussed above, most current legislation approaches the issue only from an enforcement perspective. Like illegal immigration, human trafficking is a complex, multi dimensional problem requiring multiple social service agencies' involvement. States no longer can afford to abdicate responsibility to the Federal government, nor apparently do most desire to do so, Arizona most notably. States should have the prerogative to patrol their borders, create legislation and address specific local concerns. The many state statutes presented here suggest that many jurisdictions have passed and/or are creating legislation in the addition to the Federal response. Finally, are legislative acts and legal sanctions sufficient deterrents to offset the global dire economic situations that lead to both victims (since some are driven by desperation to rely on shady contacts and desperate situations) and traffickers to engage in this activity?

This essay provides an overview of human trafficking laws as of 2009, since then some statutes may have changed. However, the will and the resources to effectively implement this legislation are also critical needs requiring empirical evaluation. This review of existing legislation begins to demonstrate the varied and diverse state responses to the problem of human trafficking.

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SELECTED PHYSIOLOGICAL RESPONSES OF KUDZU TO DIFFERENT LEVELS OF INDUCED WATER STRESS

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ABSTRACT

This study was carried out to evaluate selected responses of kudzu [*Pueraria montana* var. *lobata* (Willd.)] to various levels of stress induced by polyethylene glycol (PEG) added to hydroponic growth media. Kudzu was grown hydroponically in dissolved concentrations of PEG (0.0, 52, 79.5, and 101 g l⁻¹). The plants were grown under greenhouse conditions under the selected treatments. The objective of this study was to determine the level of stress under which kudzu could continue to grow and survive under. By determining the maximum level of osmotic stress that kudzu is able to withstand, the range of field areas to which kudzu could be adapted could then be determined. Kudzu was shown to have a significant decline in both growth and chlorophyll *a* and *b* concentration. However, carotenoid was unchanged in all of the treatments. Kudzu response to drought resulted in an induced significant decrease in the water potential of the plant and an increase in the osmotic potential. Additionally, kudzu increased the total phenolic compounds as a defense response to drought stress. The results of this study support the drought tolerance of kudzu and indicate that the plant could be introduced successfully to areas with restricted water supplies.

INTRODUCTION

The demand on food resources for both humans and livestock has increased worldwide due to the rapid increase in the human population and the destruction of the agricultural ecology of many areas. There are over 270,000 species of land plants, including 20,000 edible plants (Hammond, 1995). However, only about 20 species are utilized as the major sources of calories for human consumption. Certain species are excluded from many areas due to historical, physiological, or biotic factors. Kudzu [*Pueraria montana* var. *lobata* (Willd.)] is an under-utilized plant species with a great potential to be used for multiple purposes such as for human and animal consumption and improving soil conditions.

Native to Japan, Kudzu is a leguminous, weedy vine with pubescent stems, trifoliate leaves, and a perennial deep root system (Forseth and Teramura, 1987). Kudzu has rapid shoot growth at a rate of 29 to 30 meters per growing season and a deep tap root (Sasek and Strain, 1988). Kudzu stems spread out in all directions, with new plants beginning at stem nodes every 30 to 60 cm. The dense packing of kudzu can result in tens of thousands of plants occupying a single acre of land. Furthermore, the extensive root biomass provides high potential of exploiting deeper sources of water (Schnoor *et al.*, 1995). Moreover, kudzu can control soil erosion due to its large biomass and enhance soil fertility through nitrogen fixation (Tsugawa, 1986). Kudzu has adaptive capabilities to deal with adverse condition such as eroded soil with low fertility, low

Responses of Kudzu to Water Stress

pH, and poor water holding capacity (Lynd and Ansman, 1990). These characteristics of kudzu make it an ideal candidate to be evaluated as an alternative species for use in restoration or to protect soil from erosion. In addition, Kudzu can be considered an excellent forage crop for a variety of livestock because of its nutritional value. Kudzu contains 17.43 percent protein and, 30.2 percent starch (Kidd, 2002). Kudzu has been used in Chinese herbal medicine for over 2000 years to combat acute febrile diseases (Fang, 1980). The significance of kudzu as a medicinal plant results from its relative abundance of antioxidants (Guerra et al., 2000 and Wenli, Yaping, and Bo, 2004).

Soil erosion is major problem in arid and semiarid regions of the world. There are many factors that aggravate this problem but none more important than the absence of vegetative cover. Due to limited water availability, most of the vegetative covers disappear soon after the end of the rain season. The exceptions are the very few species with the adaptive advantage to remain in the vegetative stage. However, most of these plants are small in size and provide limited soil cover. The ultimate solution is to find a plant with relative drought resistance and a large leave area index to be introduced to these areas to protect the soil from erosion. Kudzu's rapid growth rate and large leaf area index make it an excellent plant for containing soil erosion and preventing soil damage. Introducing kudzu might be beneficial in many ways, including ecologically through its potential to reduce soil erosion and its ability to enhance soil quality. Additionally, it will provide farmers with a suitable source of feed for livestock perhaps for human consumption as well. An experiment conducted within this study to evaluate antioxidant content in kudzu, spinach (*Spinacia oleracea*), shiitake (*Lentinula edodes*), and Nori seaweed (*Porphyra* including most notably *P. yezoensis* and *P. tenera*) showed that kudzu had the highest antioxidant concentrations, exceeding spinach by 2.5 fold.

One of the major factors that determine the successful establishment of any plant species in an arid environment is the ability of the plant to endure water stress. Kudzu has the potential to exploit water deep in the soil due to its tap root (Sasek & Strain, 1988.). In a separate article, Sasek and Strain (1990) report that an increase in atmospheric carbon dioxide shows a significant decline in water potential. They predict, using a climatic model, kudzu will spread northward over one hundred kilometers due to an increase in winter temperature in association with the increase in the atmospheric carbon dioxide. However, it is not clear how kudzu responds to drought stress at the early stages of growth and the actual affect of water stress on plant growth and physiology. In this study, hydroponically grown kudzu was exposed to different levels of water stress induced artificially by dissolving different concentrations (0, 52, 79.5, and 101 g l⁻¹) of polyethylene glycol (PEG). PEG 8000 has been widely utilized in research for inducing artificial drought stress response in many plants (Steuter et al, 1981; Hardegree and Emmerich, 1990; Calamassi et al., 2001). Plant growth and photosynthetic pigments were used as indicators of kudzu's response to drought stress. Additionally, plant protective reaction was evaluated by determining the total phenols concentrations. The water potential and osmolality of the leaf were determined to evaluate the extent of the water stress in inducing these physiological responses.

METHODS AND MATERIALS

Plant material

Kudzu plants were established for this experiment by germinating kudzu seeds that were collected from naturally growing kudzu in a field near Jacksonville, AL (USA). Kudzu seeds

were scarified in concentrated sulfuric acid for 30 min. (Wechsler, 1977). The seeds were germinated in plastic pots containing a half-and-half mixture of vermiculite and potting soil. The plants were placed in a greenhouse and allowed to grow at $550 \mu\text{mol m}^{-2} \text{ s}^{-1}$ photon flux density, 45-50 % RH and air temperature of $25 \pm 5^\circ \text{C}$. Four weeks after emergence, uniform plants were individually transplanted into 900 ml glass containers, which contained half-strength Hoagland's solution (Hoagland and Arnon, 1938) at a pH of 6.5. Each container was wrapped with aluminum foil to prevent light penetration to prohibit algae growth. One week later, the plants were randomly assigned to water stress treatments. The water stress treatments were accomplished by the introduction of polyethylene glycol (PEG) to the growth media. The control was hydroponic media without polyethylene glycol. The selected concentrations of PEG were 52, 79.5, and 101 g l⁻¹. These selected PEG concentrations were calculated to induce water potential values of -0.5, -1.0, and -1.5 respectively using the following formula: $\Psi=1.22[\text{PEG}]^2T-134[\text{PEG}]^2-4.4[\text{PEG}]$, where T was 25°C (Michel, 1983). Each treatment in this study was replicated five times (5 samples). A continuous airflow through the hydroponic solution of each sample was achieved through a connection of a series of air tubes of glass containers. The plants were grown under the experimental conditions for fourteen days. At the conclusion of the experiment, individual samples were obtained from all the plants in the experiment to determine shoot water potential and osmolality. Additionally, plant growth and photosynthetic pigments were determined. The defense response of kudzu to water stress through antioxidant formation was evaluated by analyzing the plants under different treatments for anthocyanin and total phenolic compounds.

Photosynthetic pigment determination

Leaf tissue samples (0.05g) from each of the five samples of each treatment were individually placed in a vial containing 5 ml DMF (N, N-Dimethylformamide) and incubated in the dark for 36 h at 4°C to extract the photosynthetic pigments. Chlorophyll *a* and chlorophyll *b* concentrations of each sample from each treatment were determined spectrophotometrically by the method of Inskeep and Bloom (1985). Carotenoid concentrations of the DMF extract were determined spectrophotometrically at a wavelength of 470 nm and the concentrations calculated using the formula of Doong et al. (1993).

Anthocyanin content

Samples of 0.10 g fresh weight were homogenized in 5 ml of methanol containing 1% HCl (v/v). Homogenates were then filtered and anthocyanin and absorbance of the extract measured at 530 and 657 nm spectrophotometrically by the method of Mancinelli (1990).

Total Phenolic Content

The phenolic compound concentration in the leaf of kudzu was determined following a slightly modified procedure described by Singleton and Rossi (1965). Leaf tissue (3g) of each sample of each treatment was immediately frozen in liquid nitrogen and then crushed to a fine powder using a mortar and pestle. The individual samples were then extracted by stirring with 100 ml of methanol at 25°C at 150 rpm for 24 h and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 ml portions of methanol. The combined methanolic extracts were evaporated at 40°C to dryness. The total phenolic content of each extract was determined using Folin-Ciocalteau reagent and gallic acid as standard. Subsamples from each extract (50 mg) were dissolved in 25 ml of extraction solvent (40 ml acetone: 40 ml methanol: 20 ml water: 0.1 ml acetic acid) and vortexed until the extract was dissolved. The samples were then

heated at 60 °C in a water bath for 1 hour. After cooling down to room temperature, the samples were centrifuged at 1640g for 20 minutes. The supernatant was placed in a test tube and 1.0 ml of Folin-Ciocalteau was added and allowed to stand at room temperature for 5 minutes; 1.0 ml of sodium bicarbonate solution (0.566 M) was added and the tubes were vortexed and stored in the dark at room temperature for two hours. The total phenolic determination was measured spectrophotometrically at absorption of 726 nm. Gallic acid was utilized as a standard with the concentration series from 0.03-0.2 mM.

Water potential measurement

Leaf water potential of the five samples from each treatment was measured as described by Al-Hamdani *et al.* (1990). Cell sap, 100 µL, was extracted from each of the selected leaves with a French Press, loaded on a paper disc, and measured with a vapor pressure osmometer (model 5520, Wescor, Logan, UT).

Growth determination

The individual plants of each treatment were severed at the crown level, and the shoots and roots were separately oven dried at 85° C for 48 h. The shoot and root dry weights of each sample were recorded. These measurements were used to construct a shoot to root ratio for each treatment.

Data analysis

This study was repeated three times. Each replicate was represented by the different times the experiment was conducted. The data of each experiment were combined and variables were statistically analyzed as a randomized complete block design with each replicate as a block. Mean separations for the variables with significant F values, at 1 or 5 % level of probability, of the ANOVA analysis were based on the least significant difference (LSD) test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The introduction of PEG to the growth media resulted in a gradual significant decrease in both osmotic potential and water potential of kudzu leaf with the increase of the PEG concentration (Table 1). Polyethylene glycol compounds have been extensively used to induce water stress in plants grown in hydroponic media (Asadi-kavan *et al.*, 2009; Slama *et al.*, 2007). Kaufmann and Eckard (1971) reported that PEG induced water stress in plants similar to those grown in soil in drought conditions. Similarly, the reduction in water potential that is associated with the increase in PEG concentration was reported due to the reduction in the free energy of the water proportionally influenced more by metric forces than osmotic forces (Steuter, 1981). In this study, the significant decreases of both osmotic potential and water potential were expected as a common response of the plants to drought stress. The observed decrease in osmotic potential was most likely due to the accumulation of ions, inorganic and organic molecules that commonly result in reduced osmotic potential as a part of a plant's adaptive response to drought stress (Hessini *et al.*, 2009). Reduction of osmotic potential is considered as a driving force in inducing water movement from the soil into the plant to maintain turgor pressure (Blum *et al.*, 1996). Additionally, osmotic adjustment is considered as a major adaptation criteria of the plant to combat water deficit (Oliver *et al.*, 2010). A study was carried out using PEG to induce water

Table 1. Effect of different concentrations of polyethylene glycol (PEG) on water potential and osmotic potential.

PEG (g/l)	Water Potential (-MPa)	Osmotic Potential (-MPa)
0.00	0.8313a	0.75477a
52.00	0.9495b	0.86215b
79.50	1.0697c	0.97133c
101.00	1.5147d	1.37539d

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P=0.01). The LSD values were 0.0052 and 0.00469 for water potential and osmotic potential respectively.

stress in eight European provenances of *Pinus halepensis* to evaluate the water potential of the selected species to various levels of water stress (Calamassi et al., 2001). The water potential values of all the investigated pine plants were comparable to the values that were obtained in this study from kudzu leaf. The similarity in water potential response between these two studies can be used as an indicator that kudzu has the potential to osmotically adjust to combat water stress in a manner comparable to that of pine, which is considered highly adapted to arid conditions.

Water stress induced by the different PEG concentrations resulted in a significant reduction in both shoot and root growth (Table 2). However, the reduction in growth was similar at both PEG concentrations of 79.5 and 101 g l⁻¹. General observations of the plants grown at these two concentrations of PEG included the browning of the leaves, the stunting of stem growth, and rolling, which most likely resulted from the reduction in turgor pressure. The similarity in growth reduction between these two treatments could result from plants reaching the threshold of tolerance to drought at these concentration levels. The root of the plant appeared to be less sensitive to drought stress than the shoot as indicated by the lower value of shoot/root ratio of all water stress treatments in comparison to the control (Table 2). The significant

Table 2. Effect of different concentrations of polyethylene glycol (PEG) on kudzu growth.

PEG (g/l)	Shoot DW (g)	Root DW (g)	Shoot/Root Ratio
0.00	14.672a	2.905a	5.084a
52.00	7.383b	1.992b	3.704b
79.50	3.797c	1.347c	2.819c
101.00	4.861c	1.581d	3.072c

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P=0.01). The LSD values were 2.652, 0.157, and 0.756 for Shoot DW, Root DW and the Shoot/Root Ratio respectively.

reduction in the shoot/root ratio was similar at both 79.5 and 101 g/l PEG. This again could result from the stress stunting the growth of kudzu at these concentrations. In many studies, root growth was shown to continue at the expense of both leaf and stem under water stress conditions (Kramer, 1983; Westgate and Boyer, 1985; Creelman, 1990). This common response of root growth at the expense of the shoot is considered an advantage for the plant to continue water uptake and enhance the water status of the plant (Caldwell, 1976). The observed reduction in plant growth under water stress treatments is considered a common response to water stress that has been observed in many related studies (Westgate and Boyer, 1985; Burnett et al., 2006;

Jaleel et al., 2009). The impact of water stress on plant growth was interpreted as a result of a reduction in both cell enlargement and cell division (Sommer et al., 1999; Jaleel et al., 2009).

Kudzu plant subjected to water stress treatment showed a significant decline in both chlorophyll *a* and *b* in comparison to the control plants (Table 3). However, plants grown at 79.5 g/l PEG showed the most significant decrease in both chlorophylls in comparison to other water

Table 3. Effect of different concentrations of polyethylene glycol (PEG) on chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoid (Caro).

PEG (g l ⁻¹)	Chl <i>a</i> (mg g ⁻¹ FW)	Chl <i>b</i> (mg g ⁻¹ FW)	Chl <i>a/b</i> ratio	Caro(μg g ⁻¹ FW)
0.00	32.232a	28.537a	1.135a	165.376a
52.00	29.899b	22.337b	1.377ab	266.013a
79.50	21.004c	12.922c	1.625b	257.913a
101.00	24.941d	16.300d	1.533b	227.826a

Means followed by the same lowercase letter in each column are not significantly different based on the LSD test (P=0.05). The LSD values were 2.703, 6.35, 0.278, and 103.133 for chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoid (Caro) respectively.

stress treatments. The increase in chlorophyll *a* and *b* at 101 g/l PEG could be attributed to experimental errors or other unexplained reasons. However, this experiment was repeated twice with the same result. The decline in chlorophyll that was observed in this study is considered a common response of plants to water stress and has been reported in many similar studies (Sangtarash et al., 2009; Jung, 2004; Parida et al., 2007; Bacelar et al., 2006). The significantly higher ratio of chlorophyll *a/b* that was observed in plants growing at 79.5 and 101 g/l PEG indicates that chlorophyll *b* was more susceptible to these water stress treatments than was chlorophyll *a*. However, the influence of 52 g l⁻¹ PEG in the growth media on chlorophyll *a/b* was similar to that of both the control and the other water stress treatments. It appears from examining the literature that the effect of water stress on chlorophyll *a/b* ratio was mostly related to the severity of the drought stress and to the plant species. Sangtarash et al. (2009) showed higher chlorophyll *a/b* ratio in longstalk starwort plants (*Stellaria longipes*) growing in water stress conditions. Similarly, Efeoglu et al. (2009) reported that drought stress caused increases in chlorophyll *a/b* ratio in three cultivars of corn (*Zea mays*). However, drought stress induced a decline in chlorophyll *a/b* ratio in *Arabidopsis* (*Arabidopsis thaliana*) (Jung, 2004). The significance of the chlorophyll *a/b* ratio is that it influences the efficiency of photosystem II of the thylakoid membrane and can higher chlorophyll *a/b* ratio can be used as an indicator of higher drought stress tolerance (Sangtarash et al., 2009). In this study, carotenoid in kudzu leaf was shown to have a similar concentration at all the treatments (Table 3). A similar result was obtained in *Arabidopsis* in response to drought stress (Jung, 2004). However, another study that showed drought induced a significant reduction in carotenoid concentration in one of the examined wheat (*Triticum durum*) variety where as no significant difference was obtained in another variety (Loggini et al., 1999). Reduction in carotenoid concentration as influenced by water stress was shown in three varieties of corn (Efeoglu et al., 2009). These different studies support the conclusion that the response of carotenoid to water stress is dependent on plant species or the variety within the plant species in addition to the severity of the drought conditions.

The response of kudzu to water stress in this study and the responses of the various plants in the examined literature can be attributed to the impact of free radicals induced by the water stress. This presumption is supported by the findings of several studies that showed that many

physiological and biological responses, including those in this study, were negatively impacted by water stress induced free radicals (Smirnoff, 1993; Johnson et al., 2003; Nayyar and Gupta, 2006). The degree of tolerance of a plant species or a variety within a species is highly correlated with the ability of the plant to induce antioxidant response to combat the water stress induced free radicals (Smirnoff, 1993; Johnson et al., 2003). Polyphenolic compounds are considered a major antioxidant in combating free radical induced oxidative damage in the plant due to their high reactivity as hydrogen or electron donors (Manna et al, 2002; Blokhina et al., 2003). In this study, the total phenolic compound was significantly increased with the increase in water stress induced by the elevated PEG concentrations (Figure 1). This finding is supported by other studies with similar results obtained in different plant species (Parida et al., 2007; Sanchez-Rodriguez et al., 2010).

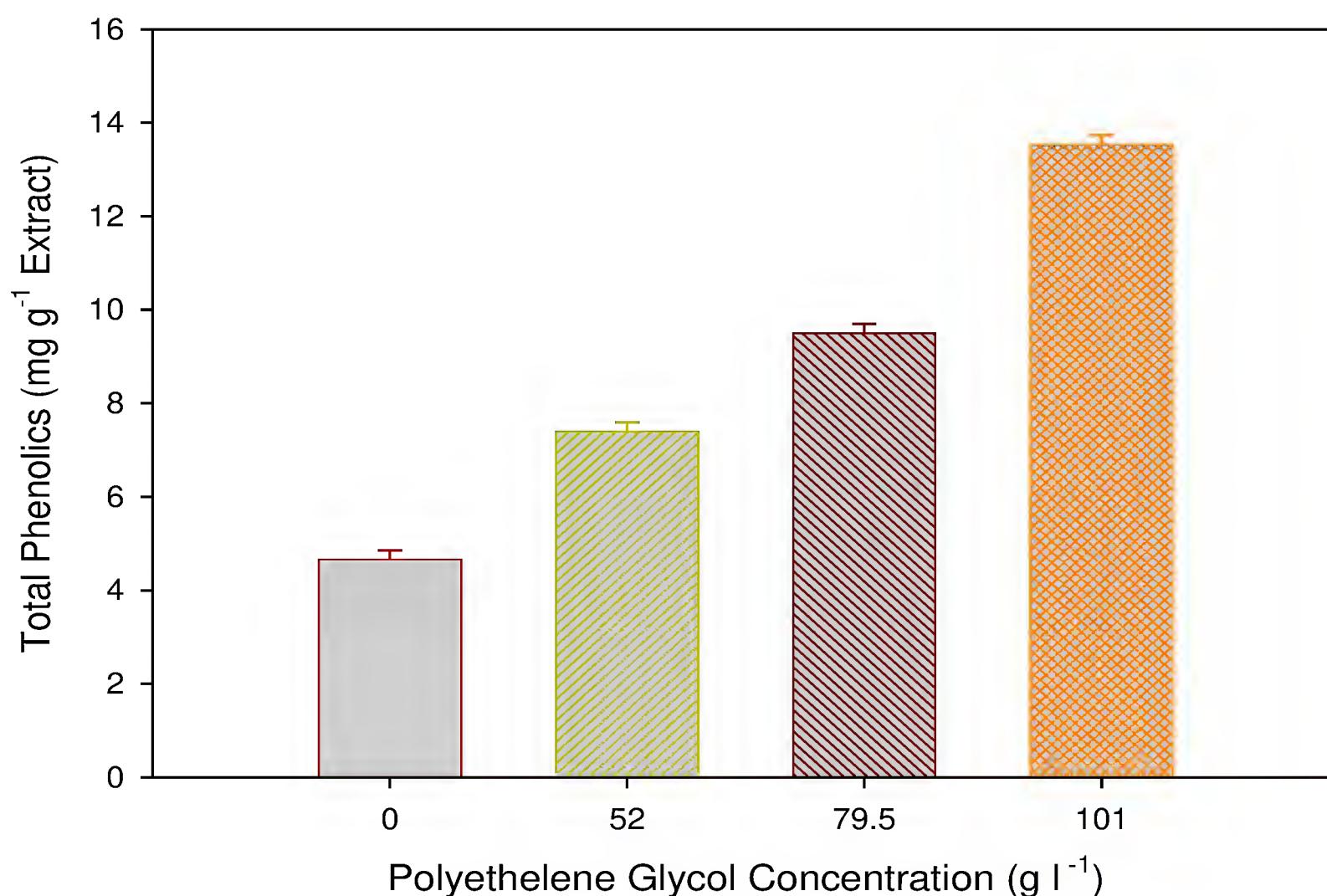


Figure 1. The Influence of different concentrations of polyethelene glycol on the total phenolic compounds of Kudzu Leaf. The LSD value was 0.823 (P=0.01).

In conclusion, induced water stress on kudzu caused a reduction in plant growth and chlorophyll concentration, factors that were selected to evaluate the response of kudzu to the selected water stress. The damage most likely resulted from the impact of free radicals induced by water stress and the alteration of water relations within the plant. However, the extent to which kudzu can avoid drought stress can be determined by the formation of polyphenolic compounds and the levels of osmotic adjustment, both of which appeared significantly increased in response to drought. Additionally, the general observation of kudzu growing in its natural habitat appeared to show high drought tolerance due to the fact that our area (Jacksonville, AL USA) endures relatively high summer temperature and extended drought periods.

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Greenish warblers live throughout Asia, as shown on the distribution map on the left. Although there's a broad range in central Siberia, the eastern Siberian & eastern Rockies (orange represented in pink) and western Siberian & northern Rockies (orange represented in orange) are not indicated.

On the map, a profound transition in color from one geographic region to another represents gene flow between adjacent populations. Notice that, despite the physical distance between them, the eastern Siberian population does exchange genes with the populations to the south. Based on the information provided, the greenish warblers consist of _____.

In 2008, Dr. Darren E. Irwin travelled all over Asia and collected data about local green warbler populations. The symbols on the map above refer to the effort he visited. These locations are represented on the graph below by a tree, under-eagle (blue), tree, under-eagle (green), tree, under-eagle (orange), and tree, under-eagle (pink). As such, he collected data on the abundance of each of these four types of trees in these areas and determined that the local environment in which a tree grows has a strong influence on bird song. For example, the abundance of the tree in a plot has an effect on how much time and effort a songbird spends singing.

How the environment varies with latitude

How bird song behavior varies with latitude

Source: Darren E. Irwin, "Song variation in an intertidal avian, *Acrocephalus fuscus*," *J. Zool.* 2008, 270: 119-126. Figure 4. Copyright © 2010.

As the amount of tree density decreases in latitude, what observations did the make about how the environment changed?

There are fewer caterpillars.

There are more caterpillars.

The habitat is more diverse.

The warblers defend themselves more.

Bird song decreases _____ decreases in latitude.

From the observational data presented, bird song in greenish warblers exhibits _____.

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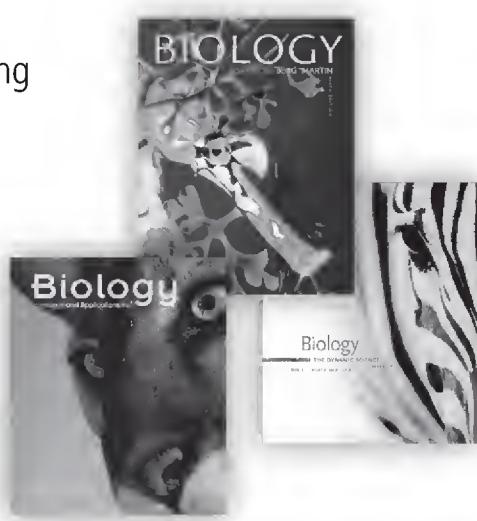
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